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PROCEEDINGS
OF THE
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CONTENTS

	PAGE
1. The Oesophagus of the Stenoglossan Prosobranchs. By Alastair Graham, M.A., B.Sc., Department of Zoology, Birkbeck College, University of London. <i>Communicated by</i> Professor Charles H. O'Donoghue. (With Five Text-figures.) Issued separately March 13, 1941,	1
*2. Non-Associative Algebra and the Symbolism of Genetics. By I. M. H. Etherington, B.A., Ph.D., Mathematical Institute, University of Edinburgh. Issued separately March 18, 1941,	24
*3. The Structure of Tulach Hill, Blair Atholl, Perthshire. By William J. McCallien, D.Sc., Carnegie Teaching Fellow, University of Glasgow. (With Four Text-figures and One Black and White Map.) Issued separately April 18, 1941,	43
4. The Hydroid of the Medusa <i>Cosmetira pilosella</i> Forbes. By W. J. Rees, Marine Biological Laboratory, Plymouth. <i>Communicated by</i> Dr A. C. Stephen. (With Two Text-figures.) Issued separately April 18, 1941,	55
*5. On <i>Cardiopteridium</i> , a Genus of Fossil Plants of Lower Carboniferous Age, with Special Reference to Scottish Specimens. By John Walton, M.A., D.Sc., Regius Professor of Botany in the University of Glasgow. (With One Plate and Three Text-figures.) Issued separately July 25, 1941,	59
6. Mutation and Lethal Effects of Ultra-Violet Irradiation on <i>Drosophila</i> . By K. Mackenzie, Ph.D., Institute of Animal Genetics, University of Edinburgh. <i>Communicated by</i> Dr P. C. Koller. (With Two Text-figures.) Issued separately July 25, 1941,	67
*7. The Genetical and Mechanical Properties of the Sex Chromosomes. VIII. The Cat (<i>Felis domestica</i>). By P. C. Koller, Ph.D., D.Sc., Institute of Animal Genetics, University of Edinburgh. (With Twenty-seven Text-figures.) Issued separately July 29, 1941,	78
8. The Structural Differentiation of Chromosome IV of <i>Drosophila simulans</i> and its Behaviour in <i>melanogaster</i> Genotype. By B. M. Slizynski, Ph.D., Institute of Animal Genetics, University of Edinburgh. <i>Communicated by</i> Dr P. C. Koller. (With Five Text-figures.) Issued separately July 29, 1941,	95
9. Cytological Analysis of Chromosome Behaviour in Three Breeds of Dogs. By I. A. Ahmed, B.Sc., Institute of Animal Genetics, University of Edinburgh. <i>Communicated by</i> Dr P. C. Koller. (With Nineteen Text-figures.) Issued separately August 8, 1941,	107

10. Geological Notes on the Stubendorff Mountains, West Spitsbergen. By W. B. Harland, Gonville and Caius College, Cambridge. *Communicated by Dr G. W. Tyrrell.* (With Three Plates and One Text-figure.) Issued separately September 22, 1941, 119
11. On the Sphincter Valve of the Antennal Gland of *Marinogammarus marinus* (Leach) sens. str. By Mary V. Schorstein, B.Sc. (Lond.). From the Department of Zoology, Victoria University, Manchester. *Communicated by Professor H. Graham Cannon, F.R.S.* (With Four Text-figures.) Issued separately October 23, 1941, 130
- *12. The Avian Ulna: Its Quill-Knobs. By George H. Edington, M.D., D.Sc., and Agnes E. Miller, M.A., Glasgow. *Communicated by Professor E. Hindle, M.A., Sc.D., Ph.D.* (With Eight Plates.) Issued separately September 16, 1941, 138
13. The Evolution of Continents: A Possible Reconciliation of Conflicting Evidence. The Bruce-Preller Lecture for 1941. By Sir Thomas H. Holland, K.C.S.I., K.C.I.E., F.R.S. Issued separately October 23, 1941, 149
14. Quantitative Characters of the Growth and Development of a Paurometabolous Insect, *Dixippus (Carausius) morosus* Br. et Redt. I. The Loss of Water in relation to Ecdysis. By Beverley N. Smallman, M.A., Ph.D., Department of Zoology, McGill University. *Communicated by Professor J. Ritchie, M.A., D.Sc.* (With Four Text-figures.) Issued separately January 30, 1942, 167
15. A Simple Apparatus for the Direct Determination of the Volume of Small Irregular Objects. By Beverley N. Smallman, M.A., Ph.D., Department of Zoology, McGill University. *Communicated by Professor James Ritchie, M.A., D.Sc.* (With One Text-figure.) Issued separately January 30, 1942, 186
- *16. Observations on Artificially Induced Heat in Immature Guinea-Pigs. By P. Bacsich, M.D., Carnegie Teaching Fellow, and G. M. Wyburn, M.B., Ch.B., D.Sc. From the Department of Anatomy, University of Glasgow. (With Two Plates.) Issued separately January 30, 1942, 188
- *17. A Study of the Cytoplasmic Inclusions during the Spermatogenesis of the Mouse. By R. A. R. Gresson, D.Sc., Department of Zoology, University of Edinburgh. *Communicated by Professor J. Ritchie, M.A., D.Sc.* (With Two Plates.) Issued separately May 5, 1942, 197
- *18. Variation in *Gryphaea incurva* (Sow.) from the Lower Lias of Loch Aline, Argyll. By Rhona M. MacIennan, B.Sc., and A. E. Trueman, D.Sc. (With Eleven Text-figures.) Issued separately May 5, 1942, 211
19. pH Phenomena in Relation to Stomatal Opening II-V. By Professor J. Small, D.Sc., Miss M. I. Clarke, and Mrs J. Crosbie-Baird, Queen's University, Belfast. (With Nine Text-figures and Two Tables.) Issued separately May 19, 1942, 233

- *20. On the Feeding and Breeding of *Calanus finmarchicus* under Laboratory Conditions. By J. E. G. Rayment, B.Sc., A.M., and F. Gross, Dr.phil.; Department of Zoology, University of Edinburgh. *Communicated by Professor James Ritchie, M.A., D.Sc. (With Two Text-figures.)* Issued separately June 23, 1942, 267
- *21. The Specific Gravity of *Calanus finmarchicus*. By F. Gross, Dr.phil., and J. E. G. Rayment, B.Sc., A.M., Department of Zoology, University of Edinburgh. *Communicated by Professor J. Ritchie, M.A., D.Sc. (With One Text-figure.)* Issued separately June 23, 1942,. 288
22. Deficiency Effects of Ultra-violet Light in *Drosophila melanogaster*. By B. M. Slizynski, Ph.D., Institute of Animal Genetics, University of Edinburgh. *Communicated by Dr A. W. Greenwood. (With Two Text-figures.)* Issued separately July 29, 1942, 297
23. Types of Development of Polytene Chromosomes. By A. M. Melland, Ph.D., John Innes Horticultural Institution, Merton, London. *Communicated by Dr P. C. Koller. (With Three Text-figures and One Plate.)* Issued separately September 24, 1942, 316
- *24. The Occurrence of Endodermis in Leguminous Root Nodules and Its Effect upon Nodule Function. By Helen L. Frazer, B.Sc., Botany Department, University of Manchester. *Communicated by Professor J. Walton, D.Sc. (With Three Text-figures.)* Issued separately October 2, 1942, 328
- *25. Growth Stages in the Ammonite *Promicroceras marstonense* Spath. By Ethel D. Currie, B.Sc., Ph.D., F.G.S. *Communicated by Professor A. E. Trueman, D.Sc., F.R.S. (With Thirteen Text-figures.)* Issued separately October, 2, 1942, 344
26. The Induction of Pregnancy in the Golden Hamster during the Breeding Pause. By O. Peczenik, M.D., Department of Zoology, University of Glasgow. *Communicated by Professor Edward Hindle, F.R.S.* Issued separately October 2, 1942, 368
27. The Larval Maxillary Glands of *Euphausia superba* Dana. By M. V. Schorstein, M.Sc., Grisedale Scholar, Department of Zoology, Victoria University, Manchester. *Communicated by Professor H. Graham Cannon, F.R.S. (With Three Text-figures.)* Issued separately February 3, 1943, . . . 375
28. Hybrid Sterility in Artificially Produced Recombinants between *Drosophila melanogaster* and *D. simulans*. By G. Pontecorvo, Institute of Animal Genetics, University of Edinburgh, and Department of Zoology, University of Glasgow. *Communicated by Professor E. Hindle, F.R.S. (With One Table and Three Text-figures.)* Issued separately February 3, 1943, . . . 385
- *29. The Effects of Radiation on Pollen Grain Development, Differentiation, and Germination. By P. C. Koller, Ph.D., D.Sc., Institute of Animal Genetics, University of Edinburgh. (With Nine Text-figures, One Plate, and Ten Tables.) Issued separately April 23, 1943, 398

	PAGE
30. Study of an Introduced North American Freshwater Mollusc, <i>Stagnicola catascopium</i> (Say). By D. Keith McE. Kevan, B.Sc., Zoology Department, University of Edinburgh. <i>Communicated by</i> Professor J. Ritchie, M.A., D.Sc. (With Ten Graphs and Twenty Text-figures.) Issued separately August 13, 1943,	430
31. Hair Pigmentation and the Genetics of Colour in Greyhounds. By Marca Burns, Zoology Department, University of Edinburgh. <i>Communicated by</i> Professor James Ritchie, M.A., D.Sc. (With One Plate.) Issued separately October 27, 1943,	462

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PROCEEDINGS

OF THE

ROYAL SOCIETY OF EDINBURGH

Section B (Biology)

VOLUME LXI

I.—The Œsophagus of the Stenoglossan Prosobranchs.

By **Alastair Graham**, M.A., B.Sc., Department of Zoology,
Birkbeck College, University of London. *Communicated by*
Professor **CHARLES H. O'DONOGHUE**. (With Five Text-figures.)

(MS. received July 24, 1940. Read November 11, 1940.)

CONTENTS.

	PAGE		PAGE
I. INTRODUCTION	I	IV. DISCUSSION	15
II. THE ŒSOPHAGUS OF THE		V. SUMMARY	21
MURICACEA	2	VI. REFERENCES TO LITERATURE	22
III. THE ŒSOPHAGUS OF THE			
BUCCINACEA	11		

I. INTRODUCTION.

THE structure of the anterior part of the alimentary canal of stenoglossan prosobranchs has been previously investigated by several workers, of whom the most important are Haller (1888) and Amaudrut (1898), although Haller's account, accurate so far as it goes, was published before there was any real knowledge of the comparative morphology of that part of the prosobranch gut. This knowledge we owe to Amaudrut, but in the case of the stenoglossan Œsophagus the description which he gives is wrong, the various parts being orientated upside down.

In the classification of Thiele (1931), which has also been adopted by Winckworth (1932), the order Stenoglossa is divided into four subdivisions—the Muricacea, Buccinacea, Volutacea, and the Toxoglossa. The specialised molluscs which belong to the last of these will not be considered in this paper; and further, I have not been able to examine personally any gastropod belonging to the Volutacea. I have, however, dissected living and preserved specimens of *Nucella* (= *Purpura*) *lapillus*

(L.) and of *Ocenebra* (= *Murex*) *erinacea* (L.) amongst the Muricacea and of *Buccinum undatum* L., *Neptunea* (= *Fusus*) *antiqua* (L.), and *Nassarius* (= *Nassa*) *reticulatus* (L.) from the Buccinacea, using the nomenclature of Winckworth (1932). The results of these investigations, which were made in Birkbeck College, University of London, and at The Marine Laboratory, Plymouth, may conveniently be given in two corresponding sections, the Muricacea being dealt with first as the more primitive.

II. THE ŒSOPHAGUS OF THE MURICACEA.

The following description of *Nucella* will apply equally, except where differences are indicated, to *Ocenebra*.

The alimentary tract of *Nucella lapillus* starts at the mouth, lying at the summit of the proboscis, which is of moderate length and of the pleurembolic type. As Amaudrut (1898) has shown, it is formed by the growth of that region of the head which lies anterior to the tentacles, and when retracted it lies inside a sheath. The mouth leads into the buccal cavity; within this is situated the odontophore bearing the radula, and into it open dorsally the ducts of the salivary glands. The common duct of a pair of accessory salivary glands opens mid-ventrally on the outer surface of the lower lip.

From the dorsal side there projects into the buccal cavity a pair of folds; these are the anterior ends of the dorsal folds of the initial portion of the prosobranch gut, and the tract between them, occupying the mid-dorsal line, is the dorsal food channel (Graham, 1932). At the posterior end of the buccal cavity the radular sac separates from the œsophagus, which is then continued backwards along the whole length of the proboscis, from the posterior end of which it runs towards the nerve ring. Using the terminology which has been previously elaborated (Graham, 1939), this part of the alimentary canal is the anterior œsophagus. When the proboscis is retracted its posterior end lies far behind the nerve ring, which has its usual position a little behind the tentacles. In order to pass through the nerve collar the anterior œsophagus has thus, on escaping from the proboscis sheath, to pass forwards on the ventral side of that structure. On reaching the region of the nerve collar it bends again backwards as described, for example, by Amaudrut (1898) for *Rapana* and by Dakin (1912) for *Buccinum*; when the proboscis is extended these bends are straightened out and the anterior œsophagus runs straight from mouth to nerve collar. When slit longitudinally the anterior œsophagus is seen (AO, fig. 1) to have longitudinally folded walls, two of the folds (DF), which occupy a ventro-lateral position, being con-

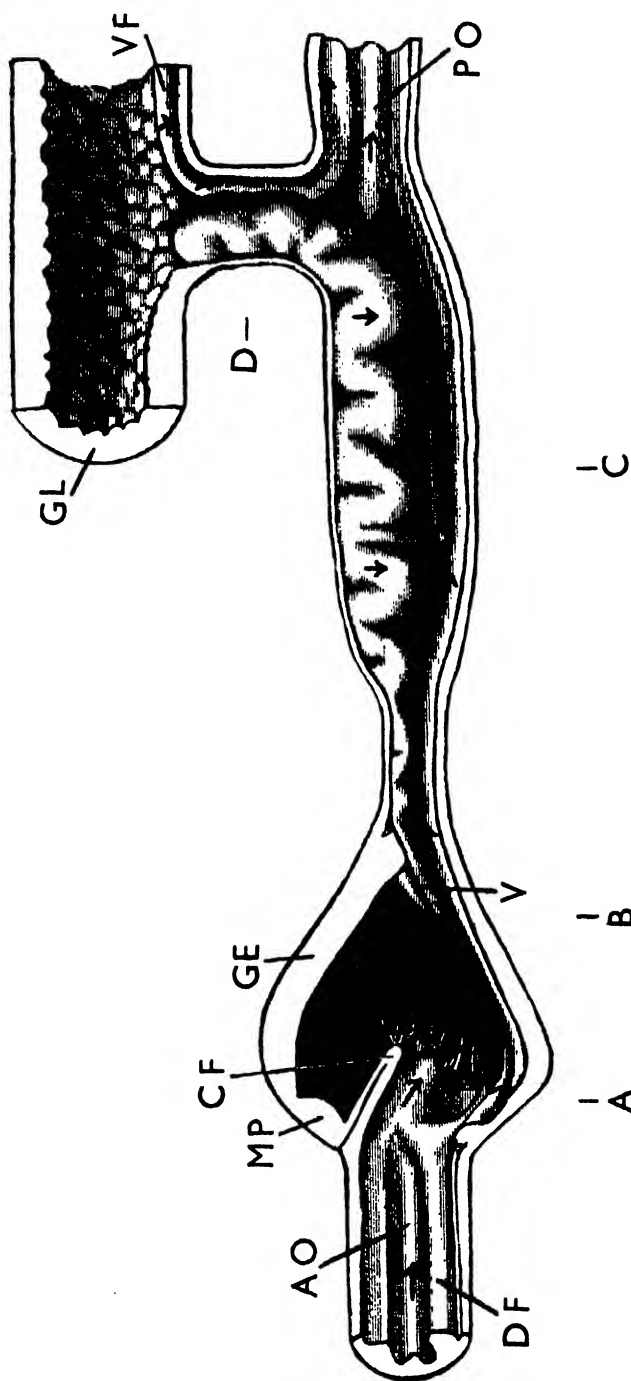


FIG. 1.—*Nucella lapillus*. A diagrammatic sagittal section of part of the Œsophagus from the posterior end of the anterior Œsophagus to the beginning of the posterior Œsophagus. Arrows show the direction of ciliary currents. A, B, C, D mark the levels of transverse sections in fig. 2. AO, anterior Œsophagus. CF, circular fold of the pharynx of Leiblein. DF, dorsal fold. GE, pad of glandular epithelium in the posterior part of the pharynx of Leiblein. GL, gland of Leiblein. MP, mucous pad. PO, posterior Œsophagus. V, cleft marking the site of the glandular part of the mid-Œsophagus. VF, ventral fold. $\times 20$.

siderably larger than any of the others. These, if traced forwards, are seen to diverge and pass dorsally, and are actually the dorsal folds. The space between them ventrally is the ventral part of the anterior œsophagus, which extends anteriorly into a triangular tongue-like lappet overhanging the mouth of the radular sac; the remainder of the walls is the dorsal food channel extended in size by the ventral migration of the dorsal folds. In the presence of recognisable dorsal folds *Nucella* appears to differ from *Rapana*, where, according to Amaudrut, there is merely a number of equally developed longitudinal folds in this part of the œsophagus.

As in other prosobranchs (e.g. *Crepidula*; Graham, 1939) the ducts of the salivary glands are embedded in the wall of the anterior œsophagus, one below each of the dorsal folds. The epithelium of this part of the gut is everywhere similar, a columnar epithelium with numerous mucous cells in the dorsal folds and food channel, bearing cilia which are especially prominent on the dorsal folds. These cilia all beat backwards.

The anterior œsophagus runs to the region of the nerve collar. Anterior to this the diameter of the alimentary canal suddenly enlarges and the œsophagus expands into a swollen pear-shaped part, with the apex of the cone directed backwards. This structure, which is variously known as the pharynx of Leiblein or the pyriform organ, lies embedded in the salivary glands, the ducts of which leave the lateral walls of the œsophagus to enter the glands just anterior to the point where the swelling begins. This swelling, as will be seen later, marks the beginning of the mid-œsophagus.

If the pyriform organ be examined from the outside a dark streak can be seen on its walls, which are elsewhere creamy white in colour. This starts anteriorly in the mid-ventral line and curves round the right side of the swelling on to the dorsal side, occupying a mid-dorsal position at the posterior end. On opening the organ by a cut which starts anteriorly in a mid-dorsal position and which curves round the left side of the gut so as to be opposite the dark line all the way, the structure of this part of the alimentary canal becomes clear. Towards the posterior end of the anterior œsophagus the two dorsal folds move towards the mid-ventral line, and at the anterior end of the pharynx of Leiblein they lie practically in contact with one another in the mid-ventral line. Separating them is an extremely narrow cleft (V, figs. 1, 2, A, 2, B), which leads between the folds into a small hernia-like protrusion of the gut possessing extraordinarily thin walls (VC, fig. 3): it is the thinness of these walls, permitting the shadowy cavity of the œsophagus to be seen through—and not the insertion of one of the dorsal folds as suggested by Amaudrut (1898)—that is the cause of the dark streak already described

as visible from the outside. The two dorsal folds and the narrow cleft between them thus rotate round the right side wall of the œsophagus and come to occupy a mid-dorsal position; this is the rotation due to the torsion of the visceral hump upon the head and foot which occurs in all prosobranchs and which is one of the principal characteristics of

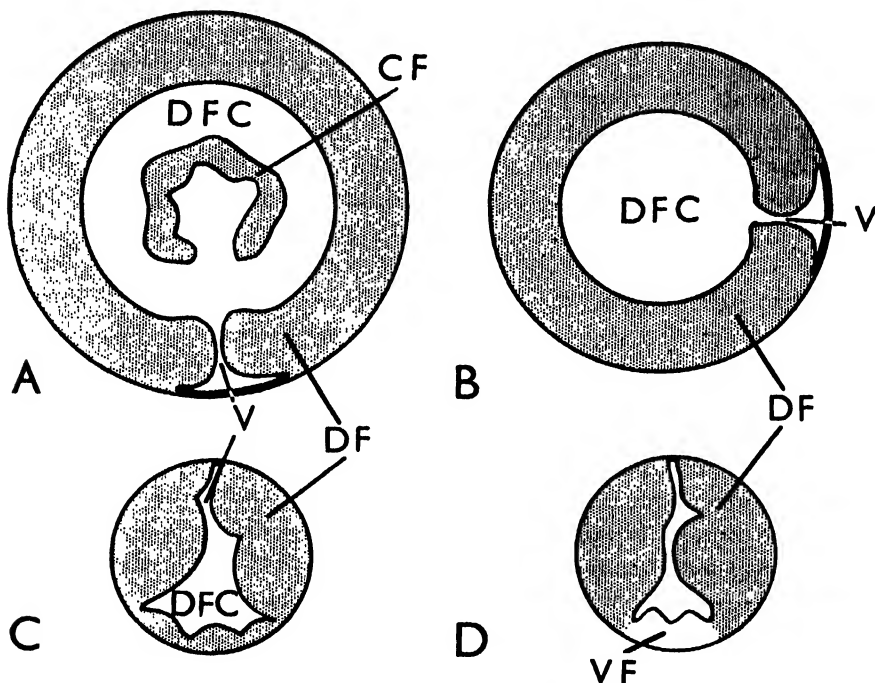


FIG. 2.—*Nucella lapillus*. Diagrammatic transverse sections through the œsophagus at the points marked in fig. 1. A, through the anterior part of the pharynx of Leiblein; B, through the posterior part of the pharynx of Leiblein; C, through the mid-œsophagus between the nerve ring and the duct of the gland of Leiblein; D, through the duct of the gland. DFC, dorsal food channel; other letters as in fig. 1. The walls of the food channel are stippled.

the mid-œsophageal region (Graham, 1939), and it has nothing to do with the over-development of the genital or other system as suggested by Amaudrut (1898). The portion of the œsophagus represented by the thin-walled cleft between the two dorsal folds, which is ventral in position in the anterior part of the pharynx of Leiblein and which is twisted round into a mid-dorsal position, is thus the homologue of the ventral glandular region of the œsophagus found in the higher Taenioglossa, and the rest of the walls of the pharynx of Leiblein represents an expansion of the dorsal food channel. This is the direct opposite of the homologies

suggested by Amaudrut (1898), who considered the pharynx an expansion of the ventral glandular part of the œsophagus.

The structure of the expanded dorsal food channel shows a certain complexity. At its anterior end a sleeve-like fold formed by a reduplication of the wall projects freely into the lumen, ending in a crenated edge (CF, figs. 1, 2, A). This fold springs from the wall all around the œsophagus except mid-ventrally, where it ends by joining the dorsal fold on each side, leaving a gap in the circle opposite the folds and the cleft between them. In *Nucella* and *Ocenebra* at least this fold is an invagination of the entire wall of the dorsal food channel and not of the right dorsal fold alone, as Amaudrut (1898) claimed to be the case in *Rapana*. Immediately behind the point of insertion of this fold the wall of the food channel is formed of a pad of mucous cells (MP) which, like the fold, runs around the dorsal food channel from one dorsal fold to the other. Behind this pad there lies a sheet of glandular epithelium (GE) which fills all the remaining part of the wall of the pyriform organ, gradually decreasing in extent as the diameter of the organ becomes smaller. It is to this sheet that the white colour of the organ is due.

The histological structure of these various parts is as follows. The dorsal folds are covered by a ciliated columnar epithelium containing occasional mucous cells just as in the anterior œsophagus. In the posterior part of the organ this passes laterally into the sheet of glandular epithelium, which consists of tall cells of two types (fig. 3). Firstly, composing the greater part of the epithelium, are columnar cells (GC), unciliated, with basal nuclei, and the cytoplasm filled with spherules of secretory material which stains darkly with iron hæmatoxylin and a bluish shade of purple with toluidin blue; secondly, alternating with these, are small wedge-shaped ciliated cells (CC), of which the bulk of the cytoplasm containing the nucleus lies near the surface of the epithelium and is connected by a long thread-like stalk to the basement membrane. The fact that the epithelium contains two kinds of cells explains the double row of nuclei described in *Concholepas* by Haller (1888) and in *Oliva* by Küttler (1913), although both these workers thought that they were dealing with an epithelium of one type of cell only. Haller's figure shows clearly that he had seen the two types, but misinterpreted what he had seen. The mucous pad lying immediately behind the line of insertion of the sleeve-like fold is also composed of two types of cells—wineglass-shaped ciliated cells with nuclei near the surface of the epithelium, alternating with elongated gland cells of which the contents give all the recognised staining reactions of mucus.

The almost circular fold which projects into the initial part of the

pharynx of Leiblein has a different type of cell on each of its surfaces. The anterior side is covered with a ciliated columnar epithelium of moderate height. It contains no gland cells of any sort, and the cells of which it is composed are only to be differentiated from the ciliated cells of the anterior Œsophagus in two points: their cytoplasm is extremely homogeneous and not vacuolated, and the cilia which they bear are

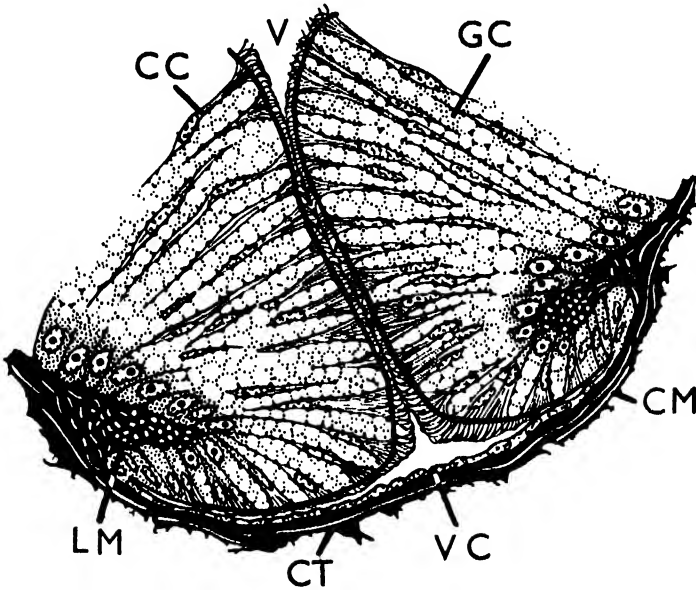


FIG. 3.—*Nucella lapillus*. Part of a transverse section through the pharynx of Leiblein showing the dorsal folds and the ventral part of the mid-œsophagus between. CC, ciliated cell. CM, circular muscle. CT, connective tissue. GC, gland cell. LM, longitudinal muscle. V, cleft between the dorsal folds. VC, cells of the ventral part of the œsophagus. $\times 280$.

much longer. As the free edge of the fold is reached the cilia steadily increase in length, and at the actual summit a sheet of very long cilia projects like a fringe into the cavity of the œsophagus. On the posterior side this fold is covered with an extension of the mucous epithelium from the pad behind, differing from that in the reduced height of the cells and in the longer cilia of the ciliated cells.

The epithelium of the morphologically ventral part, which lies hidden between the apposed dorsal folds, is a squamous epithelium of scale-like cells only $2\frac{1}{2} \mu$ thick, without gland cells or cilia (VC, fig. 3). The transition between this and the ciliated cells of the dorsal folds is abrupt. All the epithelia rest on a slight layer of connective tissue (CT), within which run a few muscle fibres mainly circular in direction (CM), although

a bundle of longitudinal muscles (LM) runs along the base of each of the dorsal folds.

The arrangement of the ciliary currents in this mid-oesophageal region is simple (fig. 1). They agree with what has been described in various other prosobranchs (Graham, 1932, 1939) in that they beat out of the morphologically ventral part of the oesophagus across the dorsal folds into the food channel, along which they beat backwards. On both sides of the circular fold the cilia beat towards the summit, where the extremely long cilia keep up a rapid trembling beat of low amplitude.

Posterior to the pharynx of Leiblein the mid-oesophagus rapidly becomes narrow in order to pass through the nerve ring. The dorsal folds in their new dorsal position draw even closer together, and the morphologically ventral region between them is reduced to a strip of cubical epithelium only three or four cells in breadth. The dorsal folds and the remainder of the food channel are occupied by an epithelium of columnar ciliated cells, in which gland cells of the type found in the posterior portion of the pharynx of Leiblein are common; ultimately these form the predominating type of cell in the dorsal folds, while the rest of the food channel is covered with ciliated cells. On the folds the cilia beat in a transverse direction into the food channel, where the currents set in a backward direction.

As soon as the narrow nerve collar has been passed the diameter of the mid-oesophagus starts to increase again, and a larger diameter is maintained as far as the point of entry of the duct of the gland of Leiblein some distance farther back. In *Nucella*, and to an even greater extent in *Ocenebra*, this stretch of the mid-oesophagus is marked by a large number of white convolutions lying on its dorsal side. These form what Haller (1888) called the "median unpaired fore-gut gland" and Amaudrut (1898) the "glande framboisée," which he regarded, wrongly, as belonging to the morphologically ventral part of the mid-oesophagus. In point of fact the convolutions are due to a folding and wrinkling of the dorsal folds themselves, and their white colour indicates the presence of great numbers of gland cells, their histological structure here being similar to what it is more anteriorly. The convolutions, as Haller showed in *Concholepas*, are arranged in a double series, lying along each side of a central furrow, and are not, as Amaudrut thought, a median structure. The two halves are formed one from each dorsal fold, the central groove from the morphologically ventral streak of cubical cells which are almost buried below the exaggerated coiling of the folds. The remainder of this part of the mid-oesophagus, lying ventrally, is not glandular like the folds, but consists of a lower ciliated epithelium containing scattered

mucous cells and thrown into longitudinal ridges. Along these ciliary currents drive particles backwards, whilst on the much-folded glandular areas of the dorsal folds they beat transversely away from the central furrow; owing to the intensely glandular nature of the epithelium covering these the currents are much weaker. Since this ventrally situated part of the mid-œsophagus is actually the dorsal food channel thrown into this position by torsion, Amaudrut's identification in *Rapana* of a fold in the mid-ventral line as the ventral fold of the mid-œsophagus is clearly wrong.

This arrangement persists until the point is reached where the duct of the gland of Leiblein enters the œsophagus on the dorsal side, running into the gland in an approximately vertical direction at a point about one-third of its total length behind the anterior end. When examined externally this duct can be seen to contain a continuation of the white convoluted mass marking the previous section of the œsophagus, and when the duct is opened the two dorsal folds, each thrown into the same series of glandular foldings as before, can be seen to pass along the anterior side of the duct with the narrow central furrow still running between them. Amaudrut (1898) denies that the folds enter the duct in *Rapana*. The longitudinal folds lying on the ventral wall of the œsophagus pass into its next section, not entering the duct of the gland. The posterior wall of the duct is occupied by a large double fold (VF, figs. 1, 2, D), the margins of which abut on the dorsal folds on each side, so that in transverse section the two dorsal folds with the morphologically ventral strip between and the double ventral fold occupy the entire wall of the duct. When the gland is reached the two dorsal glandular folds diverge slightly and then come to an abrupt end, the furrow between them enlarges and expands to form the glandular wall of the gland, and the double posterior fold bends backwards and runs along the whole of the ventral wall of the posterior part of the gland. At the œsophageal end of the duct this fold bends back and runs along the mid-dorsal side of the next section of the œsophagus. The histology of the extensions of the dorsal folds and ventral strip along the duct of the gland of Leiblein is identical with that of the same structures in the main channel of the mid-œsophagus; that of the double ventral fold is similar to what is found on the longitudinal folds in the food channel. Behind the point of entry of the duct of the gland the œsophagus (PO, fig. 1) is a narrow tube with numerous longitudinal folds on its walls, one of which, larger than the others, is the extension of that which originates in the gland. The entrance of the duct therefore marks the point at which the mid-œsophagus passes into the posterior œsophagus.

The duct of the gland of Leiblein is ciliated. Currents beat across the dorsal folds and across the double fold lying along the opposite wall of the duct. In the trough of this latter fold a strong current beats out of the gland and backwards along the œsophagus. Inside the gland itself the same arrangement of ciliary currents persists on the fold, the rest of the surface of the gland not being ciliated.

The walls of the gland are covered by an epithelium composed mainly of club-shaped cells, the cytoplasm of which is filled with large numbers of granules. These stain very intensely with Heidenhain's hæmatoxylin and with acid fuchsin, and there is very frequently a large yellow spherule lying in the swollen end of the cell. The nucleus of this type lies about the centre of the cell and is spherical; the cytoplasm can rarely be seen because of the inclusions, but in some cases a striated edge of protoplasm can be distinguished at the distal end. In addition to these occasional mucous cells may be seen, distinguished by their staining reactions and oval nuclei. The capsule of the gland is composed of connective tissue with a few muscle fibres running through it, and is very thin. The secretion of the gland is brought about by the cutting off of the tips of the first type of cell: each contains large numbers of the small darkly staining granules, and a majority also seem to possess some yellowish granules in a central vacuole. According to Hirsch (1915), the corresponding gland of *Murex trunculus* contains only a diastase; in *M. anguliferus*, on the other hand, Mansour-Bek (1934) found in it the four enzymes of the chain used for splitting the protein molecule—proteinase, carboxypolypeptidase, aminopolypeptidase, and dipeptidase.

Both *Nucella* and *Ocenebra* possess two pairs of salivary glands such as have already been described in *Rapana* and *Jopas* by Amaudrut (1898) and in several members of the Volutacea (Poirier, 1885; Woodward, 1901; Küttler, 1913). Both of these lie anterior to the nerve ring, not, as Bouvier (1887) says, one anterior and the other posterior. One pair is of compound acinous structure and lies dorsally, over the nerve ring and the pharynx of Leiblein, the right and left glands being so interdigitated as to form a single structure. The cells lining the tubules are partly ciliated, partly glandular, and the secretion appears to be mainly mucoid in nature, although both Hirsch (1915) and Mansour-Bek (1934) record the occurrence of a variety of proteolytic enzymes in the glands of species of *Murex*, and the latter is satisfied that her controls precluded the effects of autolysis. These glands, as mentioned before, discharge their secretion into the dorsal part of the buccal cavity above the tip of the odontophore, and from their position and structure are obviously homologous with the single dorsal pair of other gastropods.

The second pair of glands lies ventral and lateral to the first in the form of a coiled tube on each side. On emerging from the coil each tube runs to the middle line, ventral to the proboscis sheath, and there the two tubes unite to form a median duct of extremely narrow diameter which passes forwards to open at the tip of the lower lip. The tubules of this pair of glands are lined by unciliated columnar cells, which have dense granular protoplasm in which occasional vacuoles may be detected near the distal edges of the cells. The granules stain slightly with iron hæmatoxylin, but fairly intensely with such cytoplasmic stains as eosin. The nuclei are spherical and lie in the very bases of the cells, each with a nucleolus. The epithelium rests on a thick layer of muscle fibres, predominantly circular in direction, and outside this is a variable number of layers of polygonal cells with irregular granular contents. These can be shown to be impregnated with calcium carbonate, and it is the presence of these cells which gives the tubules the rather powdery, white appearance which they have in the living condition. The entire tubule is enclosed by a fine bounding membrane composed of squamous cells which separates it from the hæmocœl. There is no connection between the cells outside the muscle layer and those of the tubular gland within. The duct of this ventral pair of salivary glands is lined by a cubical epithelium on which cilia are patchily distributed, and is surrounded by an extension of the muscular coat which lies around the tubules.

The exact counterpart of this salivary equipment has been described in *Oliva* by Küttler (1913). The arrangement in the Muricacea is less plain, although the gross anatomy is similar in *Rapana* and *Jopas*. Haller (1888) describes only one pair of salivary glands in *Concholepas*, *Monoceros* (= *Nucella*) *muricatum* and *M. imbricatum*, but his account and the illustrations which he gives suggest that there are actually two pairs, and that he has mistaken the ventral tubular glands for parts of the ducts of the others. The genus *Murex* differs from *Ocenebra* in that only the dorsal pair is present (Bouvier, 1887; Haller, 1888; Hirsch, 1915).

III. THE ŒSOPHAGUS OF THE BUCCINACEA.

The œsophageal region of members of the Buccinacea has been described in several instances, notably in the case of *Buccinum* itself by Dakin (1912) and Brock (1936), and in a few members of the family Galeodidæ by Haller (1888), Vanstone (1894), and Mendel and Bradley (1905).

In the Buccinacea, as contrasted with the Muricacea, the first point to note in the structure of the alimentary tract is that the proboscis is

relatively longer. No accessory salivary glands are present, though the normal pair, opening to the buccal cavity, is even better developed than in the former group. The dorsal folds of the anterior œsophagus lie in their regular position in the buccal cavity and may be traced for some distance along the anterior œsophagus as it runs through the proboscis, but in the posterior section of this part of the gut they can no longer be recognised, the whole inner surface showing a large number of parallel longitudinal folds all of equal dimensions (AO, fig. 4). This arrangement leads to the initial part of the pharynx of Leiblein, which is noticeable

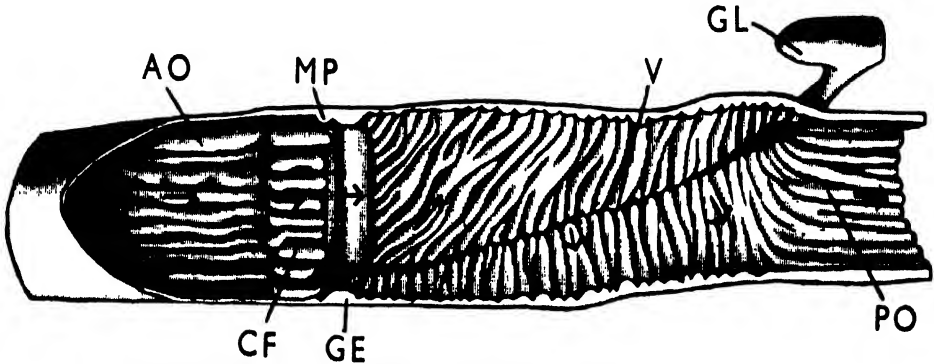


FIG. 4.—*Buccinum undatum*. A dissection of part of the œsophagus, opened a little to the left of the dorsomedian line from the posterior end of the anterior œsophagus to the beginning of the posterior œsophagus. Arrows show the direction of ciliary currents.

Letters as in fig. 1. $\times 5$.

externally as a slight swelling in the gut, but is by no means as prominent as in *Nucella*, from which it further differs in that no dark line is visible. On opening the pharynx of Leiblein it is seen that at this point each of the longitudinal folds in the more anterior section of the gut comes to an abrupt end after swelling into a keel-shaped bulge. All these structures project somewhat into the lumen of the gut to form a circular fold with lobed edges (CF). Posterior to this two cushions of epithelium separated from each other by a slight groove (MP, GE) are placed, and behind these the walls of the œsophagus show an irregular arrangement, the majority of the folds being obliquely transverse. This persists until the dorsally situated opening of the duct of the gland of Leiblein (GL) is reached, after which the œsophageal walls present a regular array of longitudinal folds (PO).

Just as the dorsal folds are less obvious in the anterior part of the œsophagus, so in the posterior section the relative extents of the dorsal food channel and of the ventral part of the gut are more difficult to determine than in *Nucella*. Anterior to the point of entry of the duct

of the gland of Leiblein, however, a distinct groove (V, figs. 4, 5) can be traced forwards as far as the posterior of the two ridges lying in the pharynx of Leiblein, cutting across the folds of the Œsophagus almost at right angles: it is shallow and not bounded by longitudinal folds. It runs along the anterior wall of the duct of the gland of Leiblein and expands at its inner end into the glandular epithelium of the gland itself. At the level of the duct of the gland the groove lies mid-dorsally, but it rotates round the right side of the Œsophagus as it passes forwards, and at the level of the posterior end of the pyriform organ it lies mid-ventrally. It is not, in any of the molluscs investigated, easy to follow the groove more anteriorly, but in *Buccinum* it can be traced over the two ridges of cells lying in the posterior half of the pharynx of Leiblein, whilst in *Neptunea* it is not only more obvious in this region, but it can also be followed across the circular fold projecting into the anterior end of that organ. On the other hand, it is difficult to find it at all in *Nassarius*. Both Dakin (1912) and Brock (1936) missed this structure in their investigations of the alimentary canal of *Buccinum*. It is obviously a reduced version of the groove representing the ventral half of the Œsophagus which occurs in *Nucella* and *Ocenebra*, the reduction affecting in the main the dorsal folds which form its lateral margins. The rest of the walls of the Œsophagus are formed by an expansion of the originally closely localised dorsal food channel. This view of the nature of the parts is supported not only by the anatomical relations of the strip, but also by the histological structure of the various parts and by the arrangement of the ciliary currents. These are shown in fig. 4, and it will be seen that as in the Muricacea the currents beat out of the morphologically ventral part into the food channel.

The histology of the various regions is very similar to what has been described above. The anterior Œsophagus, which includes that part anterior to the pharynx of Leiblein, is lined by an epithelium composed mainly of ciliated columnar cells, amongst which two types of gland cells also occur; of these one is a mucous cell, the other containing spherical secretion droplets which stain black with iron hæmatoxylin. This type of epithelium extends on to the anterior surface of the circular fold, but there it loses all the gland cells and consists entirely of cells with very finely granular cytoplasm carrying cilia which increase in length as the summit of the fold is reached. The posterior side of the fold is clothed mainly by mucous cells, between which are wedged minute ciliated cells bearing short cilia. The epithelium also covers the more anterior of the two ridges lying in the posterior half of the pharynx of Leiblein. The second of these, ring-shaped in *Buccinum* and *Nassarius*, but drawn out

into a backwardly directed extension on the dorsal side in *Neptunea*, has the same type of epithelium as covers the posterior half of the pharynx of Leiblein in *Nucella*. This does not, however, extend back to the duct of the gland of Leiblein as in that mollusc, but is replaced by an epithelium composed of fairly regularly alternating mucous (MC, fig. 5) and ciliated columnar cells (CC). The ventral part of the œsophagus (VC), represented by the groove, is covered with low unciliated cells.

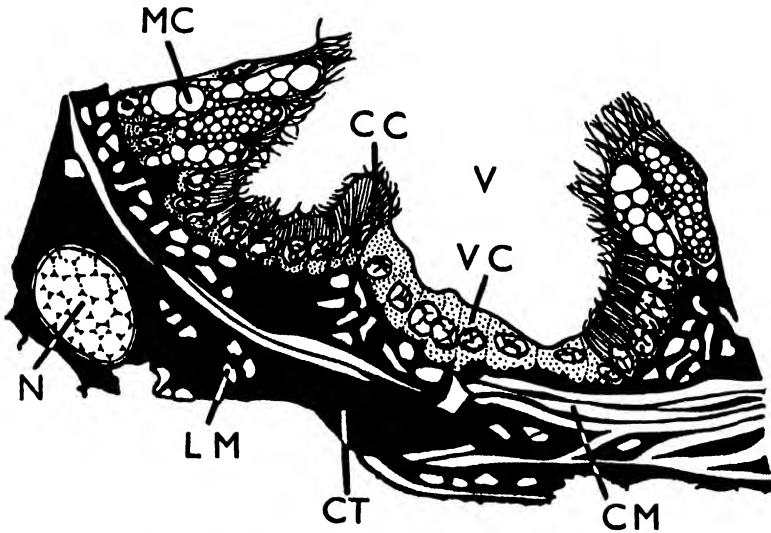


FIG. 5.—*Buccinum undatum*. Part of a transverse section through the mid-œsophagus showing the strip of cells representing the ventral part of the œsophagus. MC, mucous cell. N, nerve; other letters as in fig. 3. $\times 320$.

All rest on a basis of connective tissue (CT), through which runs a more or less regular coat of inner circular and outer longitudinal muscle fibres (CM, LM).

The gland of Leiblein of the Buccinacea differs considerably in its general appearance from the same structure in the Muricacea. The walls are much less folded, the development of gland cells is less, and the lumen of the gland correspondingly greater. Along its posterior ventral wall runs a double fold, similar to that described in *Nucella*, composed of ciliated and mucous cells. The cilia beat across each of the folds into the trough between, and along this a current drives the secretion of the gland outwards. This fold runs along the posterior side of the duct and thence backwards along the mid-dorsal side of the posterior œsophagus.

IV. DISCUSSION.

Previous work upon the œsophageal region of the *Stenoglossa* has failed to analyse the structure of the various parts involved. Most investigators have not found the narrow channel which represents, as has been shown above, the morphologically ventral part of this section of the alimentary canal, and, in the only case where any homologies have been attempted—in the work of Amaudrut (1898)—the identification of the parts which was made was unfortunately wrong. According to that author both the pharynx of Leiblein and the more posteriorly situated “glande framboisée” belonged to the morphologically ventral part; that is, they were homologous with the œsophageal pouches of the *Tænioglossa*. This homology, if correct, would have refuted a distinction which has so far proved to be of general application throughout the entire group of the prosobranchs, that the ventral part of the mid-œsophagus contains gland cells secreting enzymes, whilst the dorsal folds and the food channel between them are the site of the secretion of lubricating fluids. When histological examination showed that both the pharynx of Leiblein and the “glande framboisée” contained, almost exclusively, cells secreting mucus and other lubricants, there was at once obvious the suggestion that they must be regarded as belonging to this dorsal region, and the anatomy of the parts and the direction of the ciliary currents on them both uphold this.

Mansour-Rek (1934), it is true, has maintained that the “Blindsac,” which is composed of the exaggerated foldings of the dorsal folds, secretes proteolytic enzymes, and she records the presence of proteinase, aminopolypeptidase, and dipeptidase in extracts of the gland, carboxypolypeptidase being absent. Here is a case where the findings of biochemistry are apparently at variance with the results of comparative anatomy and histology, and I venture to suggest that in this instance it is the latter which are right, and that what Mansour-Bek has done is to demonstrate the occurrence of these enzymes in the cells of the “Blindsac” as part of the normal equipment of intracellular proteases which every cell must possess, and not as digestive enzymes secreted into the lumen of the œsophagus. But even if this second alternative should prove right the secretion of digestive enzymes by the dorsal folds is merely a further proof of the great specialisation which this part of the stenoglossan gut has undergone.

The cause of the peculiar arrangements in the œsophagus of the *Stenoglossa* must now be sought. The molluscs belonging to this group have presumably originated from a *tænioglossan* stock, probably from

somewhere close to the Doliacea. They must therefore have possessed an incipient proboscis and the normal type of tænioglossan mid-œsophagus, in which the dorsal food channel occupies about a third or quarter of the wall of the œsophagus, the rest being taken up by the glandular ventral part, which must have been separated into right and left halves by a bifid median ventral fold, of which the histological characters are the same as those of the dorsal food channel. This type of mid-œsophagus is found in *Gibbula* and has been described in *Cassis* by Amaudrut (1898).

The origin of the stenoglossan type of mid-œsophagus is due primarily to the development of a long proboscis for feeding purposes. So far as its outer wall is concerned, this is an elongation of the snout anterior to the tentacles and so to the nerve ring. Internally it causes a corresponding elongation of that part of the œsophagus which lies anterior to the nerve ring, the anterior œsophagus, and in both the Muricea and Buccinacea the entire length of the alimentary canal which lies in the proboscis behind the buccal cavity and the succeeding section, which runs to the pharynx of Leiblein, is made up of this elongated anterior œsophagus. That such is its real nature both anatomy and histology readily show. The next section, the pharynx of Leiblein itself, however, since it shows the effects of torsion, must belong to the following part of the œsophagus, the mid-œsophagus, and as it lies anterior to the nerve ring instead of posterior to that structure, the elongation of the anterior œsophagus cannot have been sufficiently great to keep pace with the increase in length of the proboscis, with the result that the anterior part of the next section of the gut has been pulled forward through the nerve ring. A similar failure on the part of the ducts of the salivary glands to grow as long as the proboscis has caused these structures too to be pulled through the nerve ring, and they now lie anterior to it, their ducts not penetrating it as they do in the lower prosobranchs. In its original tænioglossan condition, swollen into a crop, the anterior end of the mid-œsophagus would never have been able to penetrate the narrow gap formed by the nerve ring, and to allow this to take place the ventral glandular part has been stripped off the dorsal half of the œsophagus. To close the gap left by the stripping, the originally dorsally placed dorsal folds have migrated into a ventral position and now lie practically in contact with one another in the mid-ventral line, separated by a minute group of cells marking the site of attachment of the glandular ventral part. The structure of the parts is made more complex still because of the fact that the removal of the glandular ventral part cannot be limited to that portion of the mid-œsophagus which has been pulled through the nerve ring, because the posterior end of the proboscis, when retracted,

lies behind the nerve ring and dorsal to that and to the alimentary canal. This has necessitated the stripping of the glandular part off the entire length of the mid-œsophagus, so that it now communicates with that only at its extreme posterior end, at the beginning of the posterior œsophagus, where it forms the gland of Leiblein and its duct. Thus the expanded food channel now occupies, save for a microscopic morphologically ventral strip, the entire surface of the reduced mid-œsophagus. The gland of Leiblein thus represents the complete mass of the glandular ventral part, which has not, as Amaudrut (1898) suggested, been split into two parts: an anterior, represented by the pharynx of Leiblein, and a posterior, represented by the gland of Leiblein.

In a tænioglossan such as *Cassia* there runs forwards from the posterior œsophagus the bifid mid-ventral fold, which ends near the anterior limits of the mid-œsophagus. With the stripping of the ventral half of the œsophagus off the rest and its backward movement, this fold now passes along the posterior side of the duct of the gland of Leiblein and along the wall of that structure. The fold thus cannot appear on the wall of the œsophagus anterior to the entry of this duct, as was erroneously supposed by Amaudrut (1898). The fact that a median ventral fold is present at all means, too, that the Stenoglossa must have been derived from a mollusc like *Cassia*, where the fold also occurs, than *Dolium*, where the fold is lost.

The œsophageal regions of the Muricacea and of the Buccinacea agree in main points, but differ from each other in one important aspect—in that the region of the œsophagus which shows the effect of torsion in the Muricacea is the pharynx of Leiblein, lying anterior to the nerve ring, whilst in the Buccinacea it is the posterior half of the mid-œsophagus in which the rotation occurs. This fact by itself is sufficient to show that these groups represent two parallel lines of evolution and cannot be derived one from the other. Apart from this difference, the structure found in the Muricacea appears more primitive than that of the Buccinacea. In the former the dorsal folds can be traced the whole length of the œsophagus into the gland of Leiblein, which is a fairly solid gland; in the latter the identification of the dorsal folds is almost impossible, and the gland of Leiblein has become a tubular structure with a greatly decreased secreting surface. This tendency towards a reduction in the size of the gland of Leiblein and its transformation into a tubular appendage of the œsophagus appears to be continued in other members of the Buccinacea and in the Volutacea, although a considerable amount of doubt exists as to the exact arrangements in these molluscs. Among the Buccinacea the gland is distinctly less developed in *Neptunea* than

in *Buccinum*, and in the family Galeodidae Haller (1888) and Vanstone (1894) describe *Fusus* (= *Semifusus*) *probosciferus* and *Pyrula* (= *Semifusus*) *tuba* as having only a small cæcum, and *Melongena* (= *Galeodes*) *melongena* as being without a cæcum at all. In the Volutacea a tubular form again appears to be the usual form in which the gland of Leiblein is found: this is long and thick in *Neptuneopsis gilchristi* and *Voluta ancilla* (Woodward, 1901), whilst the same author describes it as thin and sac-like in *Volutilithes* (= *Volutocorbis*) *abyssicola*. A similar arrangement of the gland will probably be found to occur in *Halia priamus*, although Poirier (1887) describes the tubular appendage to this region of the œsophagus as rejoining it posteriorly so as to form what is in effect a siphon.

While it is true that the Muricacea show on the whole a more primitive arrangement than the Buccinacea, they are also more specialised in that the dorsal food channel has been elaborated to form the convoluted masses of lubricating tissue described above, and also in possessing two pairs of salivary glands, a point in which they agree with the Volutacea. An indication of incipient specialisation along similar lines can be seen in the dorsal food channel of *Gibbula* (Graham, unpublished work), where there is a broad strip of epithelium composed almost solely of mucous cells running along the inner face of each dorsal fold. This specialisation seems to be bound up with the special feeding habits of the Muricacea, which bore through the shells of worms, other molluscs or crustacea for food, whilst the Buccinacea are carrion feeders. There has long been controversy as to the exact mode of boring shells employed by these molluscs, whether chemical as in *Natica* (Schiemanz, 1891), or mechanical, and Ankel, writing in 1938, says: "Noch völlig ungeklärt ist der Bohrvorgang bei Muriciden. Wir können . . . heute noch nicht einmal sagen, wie unsere . . . Purpurschnecke bohrt, obgleich sie ohne weiteres auch im Aquarium Miesmuscheln und Strandschnecken anzufallen pflegt."

Spence Bate, writing to Forbes and Hanley (1853), expressed the belief that the boring of shells by *Nucella* could not be done by means of the radula since there was no rotatory movement during the process of boring. Most authors, however, particularly Orton (1927) and Pelseneer (1935), have regarded the boring as mechanical, although they give no details of the process. I have watched both *Nucella* and *Ocenebra* boring *Mytilus*, *Littorina*, and *Patella*, and have performed experiments on the secretion of the tubular salivary glands of the former, which, opening on the tip of the proboscis, would appear to be well situated for the production of acid if the process of boring were chemical. No acid, however, appears to be produced: the reaction of their extract in distilled

water is about pH 6.0; finely precipitated calcium carbonate in sea water or distilled water is completely unaffected by a sojourn of several days in extract of the glands, and the glands or their extract when applied to the polished inner side of a gastropod or bivalve shell leave no etched mark on the surface, as does the acid secreting gland of *Natica* (Ankel, 1937).

When a *Nucella* attacks another mollusc it contracts the foot by means of which it has attached itself to the prey, especially in the anterior region. Here the ventral surface of the foot is turned upwards, off the shell of the prey, and is retracted in the middle line so as to make the front edge of the foot crescentic, with the concavity directed forwards. Into this groove of the foot the proboscis is thrust and the antero-lateral corners of the foot then extend medianly so as to embrace the proboscis; this is thus completely invisible when a boring animal is examined by gently raising the shell. With patience, however, these parts of the foot may be made to withdraw without the process of boring being disturbed, and then it can be seen that the odontophore is in continual activity, being constantly pushed out to rasp the shell with the radular teeth. Rotation of the odontophore does take place, so that the radula is brought to bear against a fresh part of the excavation in the shell of the prey at every few strokes, but this seems to be mainly internal and affects only slightly the outer wall of the proboscis. The cavity made is more or less straight-sided, although it is basin-shaped while still unfinished. So fine is the radular scraping that it is not possible to see tooth marks on the sides of the pit. That material is being removed from the shell, however, was proved in one instance by removing a *Nucella* which had been found to be busy boring a *Mytilus* shell and examining its stomach contents: these were found to contain innumerable minute, slightly curved flakes of crystalline material which dissolved with effervescence on the addition of hydrochloric acid; these were absent from the stomachs of animals not boring. In relation with this habit of boring shells Peile (1937) has described the wearing away of the anterior radular teeth, and further support for the idea that the boring is mechanical and not chemical is derived from the fact that the mollusc will produce a hole of exactly similar size and shape if it be placed on the surface of the foot of a *Patella* as it would on boring through the shell.

As has been noted before by Piéron (1933), *Nucella*, once it has penetrated the shell of its prey, thrusts its proboscis to great lengths through the hole which it has made and feeds almost entirely on the soft tissue of the gonad and digestive gland, leaving the more solid muscles alone. The food, which breaks down easily into small particles when seized by the radula, is passed back along the anterior Œsophagus by ciliary means,

being mixed with the secretion of the salivary glands as it does so. It passes through the circular valve projecting into the pharynx of Leiblein and over the pads of gland cells filling the posterior half of that organ, and in so doing it is enclosed in a sleeve of mucus and other lubricants, in the centre of which it passes back to the level of the duct of the gland of Leiblein. Here ciliary currents bring out the secretion of the gland along the grooved ventral fold, and this is applied to the outer side of the mucous string, and the whole structure is carried back to the stomach. Lengths of this strand, consisting of central food particles and outer coatings of mucus with a line of secretion from the gland of Leiblein on one side of it, can be picked out of the posterior œsophagus and stomach.

The exact rôle of the tubular salivary glands remains obscure. I have found neither proteolytic nor amylolytic enzymes in them, and the possibility of their producing some toxic substance, suggested by the histological resemblance between them and the poison gland of *Conus* (Shaw, 1915), seems to be disposed of by the fact that their extract has no effect on the heart of *Cardium* when injected into the pericardial cavity, and by the fact that the animals which they use as prey are sedentary forms which do not require to be paralysed before they can be eaten, but which rely on their very immobility for protection. A *Patella* which has had the greater part of its viscera eaten by a *Nucella* is still capable of clinging to its home and even of moving about. As the glands do not produce acid, it seems that their function must be that of lubrication. The same applies to the normal salivary glands, though here there is probably a proteolytic enzyme secreted as in *Murex* (Mansour-Bek, 1934) and *Busycon* (Mendel and Bradley, 1905). A pedal gland which has been discovered by Fretter (1941) may also share in this function. It lies in the anterior half of the foot, opening on the ventral surface close to the anterior margin, and therefore in such a position that its aperture will be closely applied to the posterior wall of the proboscis when that is extended for boring or feeding. In females it lies just anterior to the gland which moulds the egg capsules. The secretion of this gland could thus drain down the wall of the proboscis into the excavation in the shell of the prey.

The pharynx of Leiblein acts as a valve, preventing the return into the anterior œsophagus of food which has once passed it; the importance of such a mechanism in animals which are continually elongating and contracting the anterior end of their alimentary canal during the process of feeding is obvious.

V. SUMMARY.

In the Muricea and Buccinacea the formation of a proboscis lying anterior to the nerve ring has (1) caused a lengthening of the anterior œsophagus, which also normally lies anterior to the nerve ring; (2) since this elongation was insufficient to keep up with the growth of the proboscis, pulled the anterior half of the mid-œsophagus through the nerve ring, anterior to which it now lies; (3) stripped the glandular ventral part of the mid-œsophagus off the dorsal food channel, since in its primitive condition the mid-œsophagus was too large to pass through the nerve ring.

To accommodate the retracted proboscis the stripping process has extended to the posterior part of the mid-œsophagus, so that the glandular ventral part (now called the gland of Leiblein) communicates with the mid-œsophagus only at its posterior end, at the beginning of the posterior œsophagus. To fill the gap left on the removal of the ventral part the dorsal folds have migrated ventrally and now lie apposed, joined only by a minute strip of scar tissue; the remainder of the wall of the œsophagus is an expanded dorsal food channel.

The anterior half of the mid-œsophagus now lying anterior to the nerve ring has differentiated into a glandular apparatus, the pharynx of Leiblein, which acts as a valve preventing the sucking forwards of the food when the proboscis is extended during feeding. Torsion occurs in this region in the Muricea, in the posterior half of the mid-œsophagus in the Buccinacea. In the Muricea the dorsal folds in the posterior half of the mid-œsophagus become transformed into a mass of glandular tissue secreting lubricants; they extend into the duct of the gland of Leiblein. In both groups the median ventral fold of the mid-œsophagus runs along the gland of Leiblein and its duct into the posterior œsophagus and carries the secretion of the gland into the alimentary tract.

The boring of shells by the Muricea is mechanical, done by the radula, lubricated by the tubular salivary glands and possibly by a special pedal gland, the proboscis being supported by the anterior part of the foot during the process.

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II.—Non-Associative Algebra and the Symbolism of Genetics.

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CONTENTS.

	PAGE		PAGE
§ 1. INTRODUCTION	24	§ 7. THE MENDELIAN COPULAR	
§ 2. GENETICAL MULTIPLICATION	25	ALGEBRA	32
§ 3. NON-ASSOCIATIVE PRODUCTS		§ 8. SYSTEMS OF MATING	34
AND POWERS	26	§ 9. COMPACT MULTIPLICATION	
§ 4. LINEAR ALGEBRAS	28	TABLES	37
§ 5. THE MENDELIAN GAMETIC		§ 10. OFFSPRING OF CONSAN-	
AND ZYGOTIC ALGEBRAS	29	GUINEOUS MARRIAGES	38
§ 6. "SHORTCIRCUITED" MUL-		§ 11. FURTHER GENETIC ALGEBRAS	39
TIPLICATION	31	SUMMARY	41
		REFERENCES TO LITERATURE	42

§ 1. INTRODUCTION.

THE statistical material of genetics usually consists of frequency distributions—of genes, zygotes and mating couples—from which new distributions referring to their progeny arise. Combination of distributions by random mating is usually symbolised by the mathematical sign for multiplication; but this sign is not taken literally for the simple reason that the genetical laws connecting the distributions of progenitors and progeny are inconsistent with the laws governing multiplication in ordinary algebra. This is explained more fully in § 2.

However, there is no insuperable reason why the genetical sign of multiplication should not be taken literally; for it is possible with any particular type of inheritance to construct an "algebra"—distinct from ordinary algebra but of a type well known to mathematicians—such that the laws governing multiplication shall represent exactly the underlying genetical situation. These "genetic algebras" are of a kind known as "linear algebras," of which a simple description is given in § 4.

It is not suggested that the use of ordinary algebraic methods in conjunction with the specific principles of genetics will not lead to correct results. It seems, however, that the systematic use of genetic algebras would simplify and shorten the way to their attainment, and perhaps enable much more difficult problems to be tackled with equal ease.

The construction of genetic algebras has been described in a somewhat abstract way in a previous paper (Etherington, 1939 *b*), to which I shall refer as G.A. Here I propose to consider the symbolism more from the geneticist's point of view, applying it to some simple population problems, without going into the details of the mathematical background. It will be recognised that the current treatment of such problems does in reality make use of genetic algebras without noticing them explicitly. By elaborating the symbolism and adapting it to more complicated genetical premises (*e.g.* in the manner indicated in G.A. § 14), it should be possible to avoid the laborious complexity which other methods in such cases would involve.

Only elementary mathematical knowledge is assumed, and it is hoped that this paper will be found understandable by geneticists whose mathematical knowledge is quite limited.

§ 2. GENETICAL MULTIPLICATION.

Capital letters will be used to represent frequency or probability distributions, referring to either a population, a single individual, or a single gamete; such as (in the case of autosomal allelomorphs)

$P = DD$ = homozygous dominant individual, or population consisting of such;

$P = \alpha DD + \beta DR + \gamma RR$ = population with assigned frequencies $\alpha : \beta : \gamma$ of genotypes, or individual with assigned probabilities α, β, γ of belonging to one or other genotype;

$P = \delta D + \rho R$ = population which produces D and R gametes in given numerical ratio, or gamete which has probability δ of containing D, ρ of containing R.

The *multiplication* of populations—individuals—gametes—means the calculation of progeny distribution resulting from their random mating—mating—fusion. Defining a *population* as a probability distribution of genetic types, we may say in all cases that we are multiplying populations.

Now multiplication in ordinary algebra obeys three laws: (1) the commutative law $PQ = QP$, (2) the associative law $P(QR) = (PQ)R$, (3) the distributive law $P(Q + R) = PQ + PR$.

The validity of the distributive law in the genetic symbolism is sufficiently obvious; it forms the basis of the method of "chess-board diagrams" often used as visual aids in the calculation of progeny distributions.

The associative law is not obeyed in genetical multiplication. This

is seen by comparing the progeny of a mating between the offspring from two individuals or populations, denoted as PQ, and a third individual or population R (*i.e.* the product (PQ)R), with the progeny from P and the hybrid population QR (*i.e.* the product P(QR)). There is clearly no reason why they should be the same, and in fact unless $P=R$ they are found to be different. Thus genetical multiplication is non-associative.

Regarding the commutative law, (i) if we are considering autosomal characters it will be obvious that this law applies, since the results of reciprocal matings are generally speaking identical, although we shall see below that in certain cases non-commutative multiplication can occur.

(ii) One might be tempted to say that with sex-linked characters multiplication is non-commutative, since the results of reciprocal matings are different. But it must be remembered that with sex-linked characters we can only speak of reciprocal matings in connection with the phenotype classification of a population; whereas the calculation of progeny distribution is only possible on the basis of the genotype classification. A given genotype (either involving the Y-chromosome or not) is either female or male, so that a reciprocal mating between genotypes is impossible. Suppose that we are multiplying a male genotype M and a female genotype F: then MF and FM both mean the same thing—the genotype distribution of their offspring; and so multiplication is commutative.

(iii) On the other hand, returning to autosomal inheritance, it is possible for this to be unsymmetrical in the sexes, through either crossing-over values or gametic selection being different in male and female. In such cases it is really optional whether we treat corresponding male and female genotypes as the same type (since their relevant gene content is the same) or as distinct types (since they produce different series of gametes). In the former case, PQ and QP have distinct meanings, referring to reciprocal crosses which do not produce similar distributions of offspring; and multiplication is non-commutative. In the latter case, the situation is as with sex-linkage.

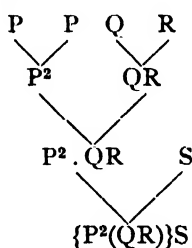
To sum up, genetical multiplication is non-associative, but obeys the commutative and distributive laws; except that in certain cases we have the option of using a varied form of the symbolism in which the multiplication is non-commutative as well as non-associative.

§ 3. NON-ASSOCIATIVE PRODUCTS AND POWERS.

Non-commutative algebra of a special kind (matrix algebra) is widely familiar by reason of its many applications in geometry and physics. (Also in genetics: *cf.* Hogben, 1933; Geppert and Koller, 1938, Chap. 4.)

Hence there is no reason to fear that an algebra which does not obey all the usual laws will necessarily prove unmanageable.

But with non-associative algebra some precautions are required to avoid confusion, especially when dealing with products or powers involving many factors. With such an expression, brackets inserted in different ways would indicate different orders of association of the factors; and the corresponding interpretations of the whole product would refer to the various pedigrees which could be constructed with given ancestors. For example, the product $\{P^2(QR)\}S$ represents the pedigree below. The



separate factors or ancestors P, P, Q, R, S may be thought of as given genotypes or distributions of genotypes. The factor P^2 may arise through self-fertilisation of an individual P , or from the mating of two individuals of the same genetic type, or from random mating within one population P or between two similar populations. The partial products $P^2, QR, P^2(QR)$, and the final result $\{P^2(QR)\}S$ are probability distributions which, for any particular type of inheritance, can be calculated when P, Q, R, S are known.

To avoid clumsiness of notation, it is convenient to use groups of dots in place of brackets, fewness of dots between factors conferring precedence in multiplication. Thus the above product would be written $P^2 \cdot QR : S$. On putting $P=Q=R=S$, it becomes a power of P . (I have discussed elsewhere a notation and nomenclature for non-associative powers (1939 *a*, § 2); *e.g.* the power in question is denoted $P^{2 \cdot 2+1}$. We shall be concerned, however, with only two simple types of non-associative powers, namely, the "principal" and "plenary" powers described below.)

Similarly, the product appearing at (10.1) below denotes

$$[(ab)(cd)(ef)][(ab)(cd)(g\dot{h})].$$

The pedigree for this is easily constructed; but it should be noted that in the context a, b, c, \dots denote gametes, so that ab, cd, \dots are the ancestral zygotes.

We shall find it important to distinguish between, *e.g.*, $P^{2 \cdot 2} = (P^2)^2$ and $P^4 = P\{P(P)\} = P : P \cdot PP$. If mating takes place at random in a popula-

tion, initially P , the successive generations, supposed discrete, are represented by the sequence of *plenary powers*

$$P, P^2, P^{2^2}, P^{2^3}, \dots, P^{2^{n-1}}, \dots, \quad (3.1)$$

each the square of the preceding; while the sequence of *principal powers*

$$P, P^2, P^3, \dots, P^n, \dots, \quad (3.2)$$

each obtained from the preceding by multiplication with P , refers similarly to a mating system in which each generation is mated back to one original ancestor or ancestral population.

§ 4. LINEAR ALGEBRAS.

Linear algebras have been studied for some ninety years, and there is an extensive literature of the subject. The following brief description will be sufficient for the present purpose. Attention is confined to algebras "over the field of real numbers"; that is to say, the Greek letters below denote ordinary real numbers, and this convention will be observed throughout the paper.

Beginning with a simple case, a *commutative* linear algebra of order 2* is determined when two given symbols or *units* A, B are subject to a multiplication table consisting of product rules of the form

$$A^2 = \alpha A + \beta B, \quad AB = \gamma A + \delta B, \quad B^2 = \epsilon A + \zeta B, \quad (4.1)$$

the coefficients being given numerical constants. The algebra then consists of all possible expressions of the form

$$P = \lambda A + \mu B, \quad (4.2)$$

which are called *hypercomplex numbers*.† Addition and multiplication of hypercomplex numbers are carried out as in ordinary algebra, the multiplication table (4.1) being used to reduce a product to the "linear" form (4.2). Thus if

$$P = \lambda A + \mu B, \quad Q = \nu A + \rho B,$$

then

$$P \pm Q = (\lambda \pm \nu)A + (\mu \pm \rho)B, \quad (4.3)$$

$$\left. \begin{aligned} PQ &= \lambda\nu A^2 + (\lambda\rho + \mu\nu)AB + \mu\rho B^2 \\ &= \lambda\nu(\alpha A + \beta B) + (\lambda\rho + \mu\nu)(\gamma A + \delta B) + \mu\rho(\epsilon A + \zeta B) \\ &= (\lambda\nu\alpha + \lambda\rho\gamma + \mu\nu\gamma + \mu\rho\epsilon)A + (\lambda\nu\beta + \lambda\rho\delta + \mu\nu\delta + \mu\rho\zeta)B. \end{aligned} \right\} \quad (4.4)$$

* *Commutative* refers to the nature of multiplication; the *order* is the number of units on which the algebra is based.

† So called because they are a generalisation of the more familiar *complex numbers*. The algebra of complex numbers possesses a *real unit* 1 and an *imaginary unit* i , which are subject to the multiplication table $1^2 = 1, i^2 = -1$.

It was implied in (4.1) that $BA = AB$. The linear algebra would be *non-commutative*, however, if different formulæ were prescribed for AB and BA ; and then we should have $PQ \neq QP$. Unless special conditions are satisfied by the coefficients in (4.1), multiplication is non-associative.

A linear algebra of order n is defined in an analogous way. It will be based on n units, and will consist of hypercomplex numbers: a hypercomplex number is an expression which is linear (*i.e.* of the first degree throughout) in the n units; and the algebra will have a multiplication table giving a linear formula for the square of each unit and for the product of each pair of units. (See, for example, the multiplication tables (5.3) and (11.10), which determine linear algebras of orders 3 and 5 respectively.)

The commutative and associative laws of addition,

$$P + Q = Q + P, \quad (P + Q) + R = P + (Q + R),$$

always hold; so do the distributive laws

$$P(Q + R) = PQ + PR, \quad (Q + R)P = QP + RP;$$

but multiplication may be non-commutative, non-associative, or both.

It will be seen that a linear algebra is completely determined when its multiplication table is known.

Given any two linear algebras of orders m and n (*i.e.* given their multiplication tables), it is possible by combining their multiplication tables in a certain way to deduce another linear algebra, of order mn , which is known as their *direct product*. This is of fundamental importance in the general theory of linear algebras, and we shall find (§ 11; *cf.* G.A. § 9) that it is also fundamental in the symbolism of genetics. If the units on which the first algebra is based are A, B, \dots , and those of the second A', B', \dots , then the units of the direct product may be interpreted as $AA', AB', BA', BB', \dots$

Also (of less importance in the mathematical theory, but equally fundamental in genetics), from any linear algebra of order n a closely related linear algebra called its *duplicate* can be derived, of order $\frac{1}{2}n(n+1)$ if the original algebra is commutative. If the original units are A, B, \dots , those of the duplicate algebra may be interpreted as A^2, B^2, AB, \dots (The process of duplication was described in G.A. § 5; *cf.* also Etherington, 1941: it occurs here in §§ 5-7.)

§ 5. THE MENDELIAN GAMETIC AND ZYGOTIC ALGEBRAS.

Consider a pair of autosomal allelomorphs D, R and the corresponding genotypes

$$A = DD, \quad B = DR, \quad C = RR. \quad \dots \quad (5.1)$$

We shall write optionally DD or D^2 , RR or R^2 . In accordance with mendelian principles and with the notation described at the beginning of § 2, we have the two sets of formulæ:

$$D^2 = D, \quad DR = \frac{1}{2}D + \frac{1}{2}R, \quad R^2 = R; \quad (5.2)$$

$$\left. \begin{array}{lll} A^2 = A, & B^2 = \frac{1}{4}A + \frac{1}{2}B + \frac{1}{4}C, & C^2 = C, \\ BC = \frac{1}{2}B + \frac{1}{2}C, & CA = B, & AB = \frac{1}{2}A + \frac{1}{2}B. \end{array} \right\} \quad (5.3)$$

These give the series of gametes produced by each type of zygote, and the series of zygotes produced by each type of mating couple, with coefficients denoting relative frequencies. *E.g.*, the second of equations (5.2) mean that a heterozygote produces D and R gametes in equal numbers; the second of equations (5.3) means that the offspring of a mating $DR \times DR$ are 25 per cent. DD, 50 per cent. DR, 25 per cent. RR.

A population P can be described by the frequencies either of the gametes which it produces, or of the zygotes which it contains, and accordingly we write:

$$(Gametic\ representation) \quad P = \delta D + \rho R, \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.4)$$

$$(Zygotic\ representation) \quad P = \alpha A + \beta B + \gamma C \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.5)$$

$$\quad \quad \quad \Rightarrow \alpha DD + \beta DR + \gamma RR, \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.6)$$

in which we may assume

$$(Normalising\ conditions) \quad \delta + \rho = 1, \quad \alpha + \beta + \gamma = 1. \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.7, 8)$$

The two representations are connected by (5.2); *i.e.* (5.6) implies (5.4) with

$$\delta = \alpha + \frac{1}{2}\beta, \quad \rho = \frac{1}{2}\beta + \gamma. \quad . \quad . \quad . \quad . \quad . \quad . \quad (5.9)$$

An examination of the above formulæ in the light of § 4 will show that by using this symbolism we are really dealing with two distinct linear algebras, both having commutative and non-associative multiplication, namely:

(1) The algebra of the symbols D, R with multiplication table (5.2). This will be called the *gametic algebra for simple mendelian inheritance*, and referred to as **G**. A hypercomplex number in this algebra has the form (5.4).

(2) The algebra of the symbols A, B, C with multiplication table (5.3). Call this the *zygotic algebra for simple mendelian inheritance*, and denote it **Z**. A hypercomplex number in **Z** has the form (5.5). Hypercomplex numbers in **G** and **Z** are interpreted as populations only if their coefficients are all positive; and it is generally convenient to require that the coefficients shall satisfy the normalising conditions (5.7, 8).

The relation between the two algebras is given by (5.1), which means that a hypercomplex number (or *linear* form) (5.5) in \mathbf{Z} is equivalent to a *quadratic* form (5.6) in \mathbf{G} . The quadratic form is reduced to a hypercomplex number in \mathbf{G} by using the multiplication table (5.2). That is to say (*cf.* 5.9), the zygotic representation determines the gametic; but not *vice versa*, owing to the extra degree of freedom in the zygotic algebra.

Starting from the gametic multiplication table (5.2), the equations (5.3) are built up by the following process: we take the symbols A, B, C defined by (5.1) as units of a new algebra, and then

$$\left. \begin{aligned} A^2 &= DD, DD = D, D = A, \\ B^2 &= (\tfrac{1}{2}D + \tfrac{1}{2}R)^2 = \tfrac{1}{4}DD + \tfrac{1}{2}DR + \tfrac{1}{4}RR = \tfrac{1}{4}A + \tfrac{1}{2}B + \tfrac{1}{4}C, \\ AB &= DD, DR = D(\tfrac{1}{2}D + \tfrac{1}{2}R) = \tfrac{1}{2}A + \tfrac{1}{2}B, \end{aligned} \right\} \quad (5.10)$$

and so on. Thus the zygotic multiplication table is constructed from the gametic. This is the process of duplication referred to in § 4, and \mathbf{Z} is thus the duplicate of \mathbf{G} .

Suppose that we wish to find the progeny distribution of two mating populations P, Q, whose representations, either gametic or zygotic, are given. We have merely to form the product of the two hypercomplex numbers; that is to say (*cf.* 4.4), we multiply two corresponding representations together as in ordinary algebra, substitute (5.2) or (5.3), and simplify. The validity of the process follows from the fact that it is simply a translation into symbols of the more self-explanatory procedure of chess-board diagrams: in other words, it follows from the fact that genetical multiplication obeys the distributive law.

§ 6. "SHORTCIRCUITED" MULTIPLICATION.

By a fundamental property of duplicate algebras (Etherington, 1941, Theorem I), multiplication in \mathbf{Z} can be "shortcircuited" by working in \mathbf{G} : that is to say, to find PQ when P and Q are given zygotically, we first apply (5.2) to obtain the gametic representations, and then multiply *without* applying (5.2). Similarly, to evaluate in \mathbf{Z} a complicated non-associative product involving any number of factors, all the operations can be performed in the simpler algebra \mathbf{G} , only the final product being left in quadratic form and interpreted as a hypercomplex number in \mathbf{Z} .

This corresponds to a well-known fact in genetics (*cf.* Jennings, 1917, pp. 101–102): in order to obtain the zygotic frequencies of an n th generation, provided that no selection acts on the zygotes, and in the absence of inbreeding, it is sufficient to trace only the gametic frequencies through the $n-1$ intervening generations.

To consider, for example, random mating of a population P *inter se*, suppose

$$P = \delta D + \rho R, \quad (6.1)$$

where (5.9) holds if the zygotic representation is given in the first place. Then the next generation is

$$\left. \begin{aligned} F_1 = P^2 &= \delta^2 DD + 2\delta\rho DR + \rho^2 RR \\ &= \alpha_1 A + \beta_1 B + \gamma_1 C, \end{aligned} \right\} \quad (6.2)$$

where

$$\alpha_1 = \delta^2, \quad \beta_1 = 2\delta\rho, \quad \gamma_1 = \rho^2. \quad (6.3)$$

This is evidently simpler than evaluating P^2 in \mathbf{Z} directly. The conclusion

$$\beta_1^2 = 4\alpha_1\gamma_1 \quad (6.4)$$

is the well-known Pearson-Hardy law.

Evaluating (6.2) in \mathbf{G} by use of (5.2),

$$\begin{aligned} P^2 &= \delta(\delta + \rho)D + \rho(\delta + \rho)R \\ &= \delta D + \rho R \end{aligned}$$

if (6.1) is normalised. Thus in \mathbf{G} any normalised hypercomplex number satisfies

$$P^2 = P; \quad (6.5)$$

hence all powers of P are equal, showing that the gene frequencies are undisturbed by random mating, or by random mating followed by any system of intermating of the generations. The zygotic distribution, however, in such cases, comes into equilibrium after one generation of random mating, since in \mathbf{Z} we find

$$P^3 = P^2, \quad P^{2,2} = P^2, \quad (6.6, 7)$$

and all higher powers of P are equal to P^2 . These equations follow immediately from (6.5) if P^3 , $P^{2,2}$ are found by short-circuited multiplication.

§ 7. THE MENDELIAN COPULAR ALGEBRA.

The procedure of duplication (5.10), by which \mathbf{Z} was derived from \mathbf{G} , can be applied to an algebra repeatedly. Let us form \mathbf{K} , the duplicate of \mathbf{Z} , and then consider its genetical significance. By analogy with (5.1) we begin by taking

$$AA, BB, CC, BC, CA, AB \quad (7.1)$$

as the units of a new algebra. There is no need to introduce fresh symbols. The multiplication table will consist of 21 equations derived by manipulation of the equations (5.3), for example:

$$(AA)^2 = AA, \quad (BB)^2 = \frac{1}{16}AA + \frac{1}{4}BB + \frac{1}{16}CC + \frac{1}{4}BC + \frac{1}{8}CA + \frac{1}{4}AB, \quad \text{etc.} \quad (7.2)$$

The interpretation is as follows: the coupled symbols (7.1) stand for the types of family into which the population can be sorted, classified according to the parental genotypes: or, we may say, they are the types of *couple* mated in the preceding generation. Hence (7.2) means that if a population of offspring of matings $A \times A$ is mated at random with itself or with a similar population, all the matings are of this type $A \times A$; but if the parental matings were all $B \times B$, then the six couple types occur in numerical proportions $\lambda^2_0 : \frac{1}{4} : \dots$; and so on.

A population for which the relative frequencies of the couple types are

$$\lambda : \mu : \nu : \theta : \phi : \psi \quad . \quad . \quad . \quad . \quad . \quad . \quad (7.3)$$

is represented by a hypercomplex number

(*Copular representation*) $P = \lambda A A + \mu B B + \nu C C + \theta B C + \phi C A + \psi A B, \quad . \quad (7.4)$

wherein

(*Normalising condition*) $\lambda + \mu + \nu + \theta + \phi + \psi = 1. \quad . \quad . \quad . \quad . \quad . \quad (7.5)$

From this we can pass by (5.3) and (5.1, 2) to the zygotic and gametic representations.

As in **G** and **Z**, the product of two hypercomplex numbers in **K** denoting populations gives in the same representation their offspring by random mating. This statement assumes that the couple types are not selected, *i.e.* they are of equal average surviving fertility; just as in **Z** and **G** we supposed no selection on zygotes or gametes. As before, multiplication in **K** can be short-circuited by working in **Z** or **G**.

Corresponding to the Pearson-Hardy law in the zygotic algebra, we have the following facts: a population, as a distribution of copular types, comes into equilibrium after *two* generations of amphimixis; after *one* generation, the equations

$$\theta^2 = 4\mu\nu, \quad \phi^2 = 4\nu\lambda, \quad \psi^2 = 4\lambda\mu \quad . \quad . \quad . \quad (7.6)$$

are satisfied; after two generations the further equation

$$\mu^2 = 16\nu\lambda \quad . \quad . \quad . \quad . \quad . \quad (7.7)$$

is satisfied; these four are the necessary and sufficient conditions for equilibrium in amphimixis, and imply also other relations such as

$$4\phi^2 = \theta\psi - \mu^2. \quad . \quad . \quad . \quad . \quad . \quad (7.8)$$

These results are obtained very simply by using short-circuited multiplication, observing that P^2 is necessarily of the form $(\alpha A + \beta B + \gamma C)^2$, and the next generation $P^{2.2}$ of the form $((\delta D + \rho R)^2)^2$.

§ 8. SYSTEMS OF MATING.

Four systems of mating will be considered. The object in each case is to obtain the distribution of types in a filial generation from the distribution in the preceding generation; also, when it can be done simply, to find the distribution in the n th filial generation, and the equilibrium distribution which this approaches as n increases. For other treatment of these and similar problems, *cf.* Jennings (1916, 1917), Wentworth and Remick (1916), Robbins (1917, 1918), Hogben (1931, Chap. 6; 1933), Geppert and Koller (1938, § 20).

(a) *Self-fertilisation, or Assortative Mating in Absence of Dominance.*

Starting from the zygotic distribution

$$P = \alpha A + \beta B + \gamma C \quad . \quad . \quad . \quad . \quad (8a.1)$$

(where $A = DD$, $B = DR$, $C = RR$), if mating proceeds in successive generations by self-fertilisation, or by each individual mating with another of the same type, the first filial generation F_1 will consist of the offspring of $A \times A$, $B \times B$, $C \times C$, occurring in proportions $\alpha : \beta : \gamma$; so that

$$F_1 = \alpha A^2 + \beta B^2 + \gamma C^2. \quad . \quad . \quad . \quad . \quad (8a.2)$$

$$= \alpha A + \beta(\frac{1}{2}A + \frac{1}{2}B + \frac{1}{2}C) + \gamma C \quad . \quad . \quad . \quad . \quad (8a.3)$$

$$= (\alpha + \frac{1}{2}\beta)A + \frac{1}{2}\beta B + (\frac{1}{2}\beta + \gamma)C. \quad . \quad . \quad . \quad (8a.4)$$

It will be seen that the frequency of heterozygotes is halved; so if the n th filial generation is denoted

$$F_n = \alpha_n A + \beta_n B + \gamma_n C, \quad . \quad . \quad . \quad . \quad (8a.5)$$

we shall have

$$\beta_1 = \frac{1}{2}\beta, \quad \beta_2 = \frac{1}{4}\beta, \quad \beta_3 = \frac{1}{8}\beta, \quad . \quad . \quad ., \quad \beta_n = \frac{1}{2^n}\beta. \quad . \quad . \quad (8a.6)$$

Also

$$\alpha_1 = \alpha + \frac{1}{4}\beta, \quad \gamma_1 = \frac{1}{4}\beta + \gamma. \quad . \quad . \quad . \quad . \quad (8a.7)$$

Let us find the quantities $u_1, u_2, u_3, . . .$ by which the hypercomplex number representing the population increases in the successive generations. We have from (8a.1) and (8a.4):

$$u_1 = F_1 - P = \frac{1}{2}\beta(\frac{1}{2}A - B + \frac{1}{2}C); \quad . \quad . \quad . \quad . \quad (8a.8)$$

and similarly we shall have

$$u_2 = \frac{1}{4}\beta(\frac{1}{2}A - B + \frac{1}{2}C) = \frac{1}{4}\beta(\frac{1}{2}A - B + \frac{1}{2}C),$$

$$u_3 = \frac{1}{8}\beta(\frac{1}{2}A - B + \frac{1}{2}C), \quad . \quad . \quad ., \quad u_n = \frac{1}{2^n}\beta(\frac{1}{2}A - B + \frac{1}{2}C). \quad . \quad (8a.9)$$

The total increase in n generations is therefore

$$u_1 + u_2 + u_3 + \dots + u_n = \left(\frac{1}{2} + \frac{1}{4} + \frac{1}{8} + \dots + \frac{1}{2^n} \right) \beta \left(\frac{1}{2}A - B + \frac{1}{2}C \right). \quad (8a.10)$$

The sum of the geometrical progression in brackets is $1 - (\frac{1}{2})^n$. Hence the n th filial generation is

$$F_n = \alpha A + \beta B + \gamma C + \left(1 - \frac{1}{2^n} \right) \beta \left(\frac{1}{2}A - B + \frac{1}{2}C \right) \quad (8a.11)$$

$$= \left(\alpha + \frac{1}{2}\beta - \frac{1}{2^{n+1}}\beta \right) A + \frac{1}{2^n}\beta B + \left(\frac{1}{2}\beta + \gamma - \frac{1}{2^{n+1}}\beta \right) C. \quad (8a.12)$$

As the number of generations increases, this quickly approaches the limiting stable distribution

$$(\alpha + \frac{1}{2}\beta)A + (\gamma + \frac{1}{2}\beta)C. \quad (8a.13)$$

(b) *Assortative Mating (Dominants \times Dominants, Recessives \times Recessives).*

The initial zygotic distribution

$$P = \alpha A + \beta B + \gamma C \quad (8b.1)$$

may be written phenotypically

$$P = (\alpha + \beta)\mathfrak{D} + \gamma C, \quad (8b.2)$$

Hence

$$\mathfrak{D} = \frac{\alpha}{\alpha + \beta}A + \frac{\beta}{\alpha + \beta}B = \frac{\alpha A + \beta B}{\alpha + \beta}, \quad (8b.3)$$

representing the genotype distribution of the dominants in P .

With the system of mating under consideration, the first filial generation is

$$F_1 = (\alpha + \beta)\mathfrak{D}^2 + \gamma C^2 \quad (8b.4)$$

$$= \frac{(\alpha A + \beta B)^2}{\alpha + \beta} + \gamma C^2. \quad (8b.5)$$

Therefore

$$\begin{aligned} (\alpha + \beta)F_1 &= (\alpha^2 A^2 + 2\alpha\beta AB + \beta^2 B^2) + (\alpha + \beta)\gamma C^2 \\ &= \alpha^2 A + 2\alpha\beta \left(\frac{1}{2}A + \frac{1}{2}B \right) + \beta^2 \left(\frac{1}{4}A + \frac{1}{2}B + \frac{1}{4}C \right) + (\alpha + \beta)\gamma C \\ &= (\alpha^2 + \alpha\beta + \frac{1}{4}\beta^2)A + (\alpha\beta + \frac{1}{2}\beta^2)B + (\frac{1}{4}\beta^2 + \alpha\gamma + \beta\gamma)C. \end{aligned} \quad (8b.6)$$

It will be found that $F_1 - P$ is a multiple of $\frac{1}{2}A - B + \frac{1}{2}C$, and hence that F_n can be found by summation of a series, just as in Case (a). The series in this case is not a geometrical progression, but it is of a type whose sum is easily obtained. Following the procedure of Case (a), it will be found that the total increase in n generations can be expressed as

$$\begin{aligned}
 & \left\{ \frac{\frac{1}{2}\beta}{(a+\frac{1}{2}\beta)(a+\beta)} + \frac{\frac{1}{2}\beta}{(a+\beta)(a+\frac{3}{2}\beta)} + \dots + \frac{\frac{1}{2}\beta}{(a+\frac{1}{2}n\beta)(a+\frac{1}{2}(n+1)\beta)} \right\} \beta(a+\frac{1}{2}\beta)(\frac{1}{2}A-B+\frac{1}{2}C) \\
 &= \left\{ \left(\frac{1}{a+\frac{1}{2}\beta} - \frac{1}{a+\beta} \right) + \left(\frac{1}{a+\beta} - \frac{1}{a+\frac{3}{2}\beta} \right) + \dots + \left(\frac{1}{a+\frac{1}{2}n\beta} - \frac{1}{a+\frac{1}{2}(n+1)\beta} \right) \right\} \beta(a+\frac{1}{2}\beta)(\frac{1}{2}A-B+\frac{1}{2}C) \\
 &= \left\{ \frac{1}{a+\frac{1}{2}\beta} - \frac{1}{a+\frac{1}{2}(n+1)\beta} \right\} \beta(a+\frac{1}{2}\beta)(\frac{1}{2}A-B+\frac{1}{2}C) \\
 &= \left\{ \beta - \frac{\beta(a+\frac{1}{2}\beta)}{a+\frac{1}{2}(n+1)\beta} \right\} (\frac{1}{2}A-B+\frac{1}{2}C). \quad \dots \quad (8b.7)
 \end{aligned}$$

The n th filial generation is obtained by adding (8b.7) to (8b.1). We obtain

$$\begin{aligned}
 F_n &= aA + \beta B + \gamma C + \beta(\frac{1}{2}A-B+\frac{1}{2}C) - \frac{\beta(a+\frac{1}{2}\beta)}{a+\frac{1}{2}(n+1)\beta} (\frac{1}{2}A-B+\frac{1}{2}C) \\
 &= (a+\frac{1}{2}\beta)A + (\frac{1}{2}\beta+\gamma)C - \frac{\beta(a+\frac{1}{2}\beta)}{a+\frac{1}{2}(n+1)\beta} (\frac{1}{2}A-B+\frac{1}{2}C). \quad \dots \quad (8b.8)
 \end{aligned}$$

As n increases, the fraction with $a+\frac{1}{2}(n+1)\beta$ in the denominator approaches zero. Hence F_n approaches a stable distribution, namely, $(a+\frac{1}{2}\beta)A + (\frac{1}{2}\beta+\gamma)C$, the same as in Case (a). (Cf. 8a.13.)

(c) Fraternal Mating.

In this and the following case it is necessary to use the copular representation (7.4), from which of course the zygotic representation can be deduced. The determination of F_n is much more difficult than in Cases (a) and (b). It is best obtained with the aid of matrix algebra; and as this is beyond the scope of this paper, I content myself with showing only in each case how the copular representation of any generation is deduced from the preceding.

Suppose that initially

$$P = \lambda AA + \mu BB + \nu CC + \theta BC + \phi CA + \psi AB, \quad \dots \quad (8c.1)$$

and that brothers and sisters are mated at random. Then the filial generation is

$$F_1 = \lambda(AA)^2 + \mu(BB)^2 + \nu(CC)^2 + \theta(BC)^2 + \phi(CA)^2 + \psi(AB)^2. \quad \dots \quad (8c.2)$$

Using short-circuited multiplication (*i.e.* (5.3) instead of (7.2)),

$$\begin{aligned}
 F_1 &= \lambda(A)^2 + \mu(\frac{1}{2}A + \frac{1}{2}B + \frac{1}{2}C)^2 + \nu(C)^2 + \theta(\frac{1}{2}B + \frac{1}{2}C)^2 + \phi(B)^2 + \psi(\frac{1}{2}A + \frac{1}{2}B)^2 \\
 &= \lambda AA + \mu(\frac{1}{4}AA + \frac{1}{4}BB + \frac{1}{4}CC + \frac{1}{4}BC + \frac{1}{4}CA + \frac{1}{4}AB) + \nu CC \\
 &\quad + \theta(\frac{1}{4}BB + \frac{1}{2}BC + \frac{1}{4}CC) + \phi BB + \psi(\frac{1}{4}AA + \frac{1}{2}AB + \frac{1}{4}BB) \\
 &= (\lambda + \frac{1}{4}\mu + \frac{1}{4}\psi)AA + (\frac{1}{4}\mu + \frac{1}{2}\theta + \phi + \frac{1}{4}\psi)BB + (\frac{1}{4}\mu + \nu + \frac{1}{4}\theta)CC \\
 &\quad + (\frac{1}{4}\mu + \frac{1}{2}\theta)BC + \frac{1}{4}\mu CA + (\frac{1}{4}\mu + \frac{1}{2}\psi)AB. \quad \dots \quad (8c.3)
 \end{aligned}$$

(d) *Filial Mating.*

Starting from an arbitrary copular distribution as in (c), suppose that each individual (or each individual of one sex) is mated with the parent of opposite sex. Then

$$\begin{aligned}
 F_1 &= \lambda AA.A + \mu BB.B + \nu CC.C + \theta BC.(\tfrac{1}{2}B + \tfrac{1}{2}C) + \phi CA.(\tfrac{1}{2}C + \tfrac{1}{2}A) \\
 &\quad + \psi AB.(\tfrac{1}{2}A + \tfrac{1}{2}B) \quad (8d.1) \\
 &= \lambda A.A + \mu(\tfrac{1}{2}A + \tfrac{1}{2}B + \tfrac{1}{2}C)B + \nu C.C + \theta(\tfrac{1}{2}B + \tfrac{1}{2}C)^2 + \phi B(\tfrac{1}{2}C + \tfrac{1}{2}A) + \psi(\tfrac{1}{2}A + \tfrac{1}{2}B)^2 \\
 &= \lambda AA + \mu(\tfrac{1}{4}AB + \tfrac{1}{4}BB + \tfrac{1}{4}BC) + \nu CC + \theta(\tfrac{1}{4}BB + \tfrac{1}{4}BC + \tfrac{1}{4}CC) \\
 &\quad + \phi(\tfrac{1}{4}BC + \tfrac{1}{4}AB) + \psi(\tfrac{1}{4}AA + \tfrac{1}{4}AB + \tfrac{1}{4}BB) \\
 &= (\lambda + \tfrac{1}{4}\psi)AA + (\tfrac{1}{2}\mu + \tfrac{1}{4}\theta + \tfrac{1}{4}\psi)BB + (\nu + \tfrac{1}{4}\theta)CC + (\tfrac{1}{4}\mu + \tfrac{1}{2}\theta + \tfrac{1}{4}\phi)BC \\
 &\quad + o.CA + (\tfrac{1}{4}\mu + \tfrac{1}{2}\phi + \tfrac{1}{4}\psi)AB. \quad . \quad . \quad (8d.2)
 \end{aligned}$$

§ 9. COMPACT MULTIPLICATION TABLES.

If P and Q are any two normalised hypercomplex numbers in \mathbf{G} (say $\delta D + \rho R$, $\delta'D + \rho'R$, where $\delta + \rho = \delta' + \rho' = 1$), then

$$PQ = \tfrac{1}{2}P + \tfrac{1}{2}Q. \quad . \quad . \quad . \quad (9.1)$$

This may be shown directly by multiplying and applying (5.2); or more briefly by observing that $\tfrac{1}{2}P + \tfrac{1}{2}Q$ is also normalised, so that by (6.5)

$$\begin{aligned}
 P^2 &= P, \quad Q^2 = Q, \\
 \tfrac{1}{2}P + \tfrac{1}{2}Q &= (\tfrac{1}{2}P + \tfrac{1}{2}Q)^2 = \tfrac{1}{4}P^2 + \tfrac{1}{2}PQ + \tfrac{1}{4}Q^2 = \tfrac{1}{4}P + \tfrac{1}{2}PQ + \tfrac{1}{4}Q,
 \end{aligned}$$

from which (9.1) follows.

The result (9.1) may be regarded as a compact form of the gametic multiplication table, since it includes the three equations (5.2) as special cases. (It must be noted that (9.1) only applies if P, Q are normalised. The more general result is: $PQ = \tfrac{1}{2}(\delta' + \rho')P + \tfrac{1}{2}(\delta + \rho)Q$.)

It will be convenient to use the letters a, b, c, \dots to denote each either D or R, and then the compact multiplication table may be written

$$ab = \tfrac{1}{2}a + \tfrac{1}{2}b. \quad . \quad . \quad . \quad (9.2)$$

Applying to this the process of duplication, we obtain

$$\begin{aligned}
 ab.cd &= (\tfrac{1}{2}a + \tfrac{1}{2}b)(\tfrac{1}{2}c + \tfrac{1}{2}d), \\
 i.e. \quad ab.cd &= \tfrac{1}{4}ac + \tfrac{1}{4}ad + \tfrac{1}{4}bc + \tfrac{1}{4}bd, \quad . \quad . \quad . \quad (9.3)
 \end{aligned}$$

which gives a compact form of the zygotic multiplication table: for it includes all the six equations (5.3) as special cases. *E.g.* on putting $a=c=D$, $b=d=R$, we get from (9.3) the formula for B^2 .

§ 11. FURTHER GENETIC ALGEBRAS.

Consider inheritance depending on two pairs of autosomal allelomorphs, say D, R and D', R'. The corresponding gametic algebras **G**, **G'** have multiplication tables:

$$\left. \begin{array}{lll} D^2 = D, & DR = \frac{1}{2}D + \frac{1}{2}R, & R^2 = R; \\ D'^2 = D', & D'R' = \frac{1}{2}D' + \frac{1}{2}R', & R'^2 = R'. \end{array} \right\} \quad (11.1)$$

Taking both pairs into account, there are four gametic types:

$$DD', \quad DR', \quad RD', \quad RR', \quad . \quad . \quad . \quad (11.2)$$

whose multiplication table is constructed as follows:—

$$\left. \begin{array}{l} DD'.DD' = D^2, D'^2 = DD', \\ DD'.DR' = D^2, D'R' = D(\frac{1}{2}D' + \frac{1}{2}R') = \frac{1}{2}DD' + \frac{1}{2}DR', \\ DR'.RD' = DR, D'R' = (\frac{1}{2}D + \frac{1}{2}R)(\frac{1}{2}D' + \frac{1}{2}R') = \frac{1}{4}DD' + \frac{1}{4}DR' + \frac{1}{4}RD' + \frac{1}{4}RR', \end{array} \right\} \quad (11.3)$$

and so on. (10 equations.)

(It will be seen that although multiplication is non-associative we assume, *e.g.*, $DD'.DR' = D^2.D'R'$. This is justified because the combination of dashed and undashed symbols is mere juxtaposition, not genetical multiplication.) This is precisely the process referred to in § 4 of forming the direct product of the two algebras **G**, **G'**, which is well known in the theory of linear algebras.

Alternatively, let us use *a*, *b* to denote each either D or R, and *a'*, *b'* similarly for D' or R', so that, for example, *aa'* can denote any of the four gametic types. Then we can write the joint multiplication table in the compact form:

$$aa'.bb' = ab.a'b' = (\frac{1}{2}a + \frac{1}{2}b)(\frac{1}{2}a' + \frac{1}{2}b'), \quad (11.4)$$

i.e.

$$aa'.bb' = \frac{1}{4}aa' + \frac{1}{4}ab' + \frac{1}{4}ba' + \frac{1}{4}bb'. \quad (11.5)$$

The zygotic algebra is obtained by duplicating the gametic, and is the direct product **ZZ'**. That is to say, it is immaterial whether the process of duplication is carried out before or after that of forming the direct product (Etherington, 1941, Theorem V). There is one point, in this connection, which requires elucidation. It has been pointed out that by pairing the four gametic types (11.2) we obtain the ten types of zygote, namely:

$$\begin{array}{l} DD'.DD', \quad DD'.DR', \quad DD'.RD', \quad DD'.RR', \quad DR'.DR', \quad DR'.RD', \\ DR'.RR', \quad RD'.RD', \quad RD'.RR', \quad RR'.RR', \end{array} \quad (11.6)$$

which figure in (11.3). There are, however, only nine genotypes, namely:

$$\begin{array}{l} DDD'D', \quad DDD'R', \quad DDR'R', \quad DRD'D', \quad DRD'R', \quad DRR'R', \quad RRD'D', \\ RRD'R', \quad RRR'R', \end{array} \quad (11.7)$$

there being no distinction between the double heterozygotes $DD' \cdot RR'$ and $DR' \cdot RD'$, which both give rise to the genotype $DRD'R'$. The fact is that for calculating progeny distributions it is really optional whether we use the *zygotic algebra* based on the ten zygotic types (11.6), or the *genotypic algebra* based on the nine genotypes (11.7). The latter is obtained from the former simply by suppressing the distinction between $DD' \cdot RR'$ and $DR' \cdot RD'$. To geneticists the genotypic algebra would seem to be the obvious one to use; but the zygotic algebra is mathematically much simpler—firstly, because it is a direct product of simpler algebras; secondly, because it is a duplicate algebra; thirdly, because its multiplication table can be written in the compact form (11.5). In the final interpretation of any results obtained by use of the zygotic algebra, the distinction between the equivalent double heterozygotic types can be suppressed; just as with any zygotic or genotypic algebra, in the final interpretation of any calculation, the distinction between genotypes which are the same phenotype may be dropped in order to obtain a result true for phenotypes.

Some genetic algebras representing more complicated types of symmetrical inheritance were considered in G.A., including (§ 14) a group of three linked series of multiple allelomorphs, and (§ 15) inheritance in tetraploids. These algebras can all be manipulated on the lines illustrated for simple mendelian inheritance, the extra complication being to some extent offset by the consistent use of compact multiplication tables. As long as only symmetrical inheritance is considered, and zygotic types (differing in their gametic formation) are used rather than genotypes (differing in their relevant gene content), the corresponding gametic, zygotic and copular algebras are related by duplication; and two or more independent genetic algebras of the same kind (**G**, **Z** or **K**) can be combined by forming their direct product.

Let us finally consider briefly an *unsymmetrical* genetic algebra, *i.e.* representing inheritance which is not symmetrical in the sexes.

Consider a single gene difference D, R on the X-chromosome in a species where the male is heterogametic. The gametic types are

$$\text{Ova, } D, R; \quad \text{Sperm, } D, R, Y; \quad . \quad . \quad . \quad (11.8)$$

and the zygotic types with the gametes which they produce give the multiplication rules:

$$\left. \begin{array}{l} \text{Female, } a \equiv DD = D, \quad b \equiv DR = \frac{1}{2}D + \frac{1}{2}R, \quad c \equiv RR = R; \\ \text{Male, } d \equiv DY = \frac{1}{2}D + \frac{1}{2}Y, \quad e \equiv RY = \frac{1}{2}R + \frac{1}{2}Y. \end{array} \right\} \quad (11.9)$$

Hence $ad = D(\frac{1}{2}D + \frac{1}{2}Y)$, $bd = (\frac{1}{2}D + \frac{1}{2}R)(\frac{1}{2}D + \frac{1}{2}Y)$, and so on; whence we have the zygotic multiplication table:

$$\left. \begin{aligned} ad &= \frac{1}{2}a + \frac{1}{2}d, & bd &= \frac{1}{4}a + \frac{1}{4}b + \frac{1}{4}d + \frac{1}{4}c, & cd &= \frac{1}{2}b + \frac{1}{2}c, \\ ae &= \frac{1}{2}b + \frac{1}{2}d, & be &= \frac{1}{4}b + \frac{1}{4}c + \frac{1}{4}d + \frac{1}{4}e, & ce &= \frac{1}{2}c + \frac{1}{2}e. \end{aligned} \right\}$$

Since two males or two females produce no offspring, we must write also

$$a^2 = b^2 = c^2 = d^2 = e^2 = ab = bc = ca = de = 0.$$

It will be seen that the zygotic algebra is not obtained entirely by the process of duplication, since this would give, *e.g.*, $a^2 = a$. If a population is denoted

$$P = aa + \beta b + \gamma c + \delta d + \epsilon e, \quad (11.11)$$

and the male and female components are normalised separately:

$$a + \beta + \gamma = \delta + \epsilon = 1, \quad (11.12)$$

and if Q is another population represented in the same manner, then the product PQ describes the population of offspring, and will be automatically normalised.

An equally satisfactory scheme is to write instead of (11.9)

$$\left. \begin{aligned} a &\equiv DD = D, & b &\equiv DR = \frac{1}{2}D + \frac{1}{2}R, & c &\equiv RR = R, \\ d &\equiv DY = D + Y, & e &\equiv RY = R + Y, \end{aligned} \right\} \quad (11.13)$$

giving

$$ad = a + d, \quad bd = \frac{1}{2}a + \frac{1}{2}b + \frac{1}{2}d + \frac{1}{2}e, \quad \text{etc.} \quad (11.14)$$

We deal in this case with the female and male components of a population separately:

$$F = aA + \beta b + \gamma c, \quad M = \delta d + \epsilon e. \quad (\alpha + \beta + \gamma = \delta + \epsilon = 1.) \quad (11.15)$$

The offspring by random mating is given by their product, separated similarly into two components.

The numerical coefficients which appear on the right sides of (11.14) correspond to the asymmetrical extensors of Hogben's matrix notation (1933), just as the coefficients in (5.3) correspond to the symmetrical extensors.

SUMMARY.

The sign \times is used by geneticists to indicate crossing of types. Literal interpretation of this as a symbol of multiplication leads to a type of algebra in which the associative law $P \times (Q \times R) = (P \times Q) \times R$ is not obeyed. The different "algebras" which in this way correspond to the various possible modes of inheritance known in genetics are therefore necessarily different from the algebra of ordinary numbers. They are of a kind known as "linear algebras," and it is shown that various genetical problems can be conveniently treated by means of a symbolism based on this fact.

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III.—The Structure of Tulach Hill, Blair Atholl, Perthshire.

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and White Map.)

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INTRODUCTION.

1. Tulach Hill (1537 ft.) rises steeply from the southern side of the River Garry, about a mile to the south-west of Blair Atholl (fig. 1). It is the culminating point of a low range of hills running north-west from the confluence of the rivers Garry and Tummel and lying between Strath Garry on the north and Strath Tummel and Fincastle Glen on the south. To the west, the Tulach ridge descends in a drift-covered slope into the valley of Allt Bhaic, a northward flowing tributary of the Garry with its source in Loch Bhac.

The country to the west of Allt Bhaic is undulating moorland, typical of the Struan Flags (Moine Gneiss), and lies outside the scope of this paper.

2. Tulach Hill was originally mapped for the Geological Survey by J. S. Grant Wilson and is included in Sheet 55 of the Geological Survey One-inch Map of Scotland. According to Grant Wilson the whole of the area between the Loch Tay fault and Garry Bridge (fig. 1) is composed of complexly folded quartzite, quartz-schist, black schist, and limestone, with minor outcrops of epidiorite. The quartzite and quartz-schist were interpreted by Grant Wilson and Cunningham Craig (1905, pp. 8-61; 1912, p. 26) as being younger than the limestone (Blair Atholl Limestone), which in turn was believed to overlie the black schist.

Barrow's interpretation of the stratigraphy, however, stressed the "two-sided" or intercalatory nature of the quartzite, with the Blair Atholl Limestone as a later, sometimes unconformable, horizon.

Fortunately, we do not need to discuss the merits of these and other interpretations of the stratigraphy for the matter has already been dealt with by Bailey, who, in 1925, demonstrated the essential truth of Barrow's interpretation of the intercalated quartzite, at the same time dividing

the black schist into two distinct stratigraphical members, the Blair Atholl Dark Schist, older than the Blair Atholl Limestone, and the Ben Eagach Black Schist, younger than the Perthshire Quartzite Series. Bailey also demonstrated that much of the country which was mapped as black schist and quartzite was composed of a group of essentially non-graphitic mica-schists and quartz-schists, to which he gave the name Killiecrankie Schist. This forms the middle division of the Perthshire Quartzite Series.

In 1931, Gregory put forward a still different interpretation, according to which part of the Perthshire Quartzite Series, namely its best known member, the Schiehallion Quartzite, was placed above the Blair Atholl Series, and part, namely the Carn Mairg Quartzite (Cammoch Hill Quartzite) and the Killiecrankie Schist, was placed below the Blair Atholl Limestone, which in turn was believed by Gregory to be older than the Blair Atholl Dark Schist. Gregory's stratigraphical table, however, was based on mistaken readings of the exposures, although he had earlier (1910, pp. 7-10) anticipated Bailey in pointing out the existence of two distinct quartzites in the Perthshire Quartzite Series.

The stratigraphical classification now in use may be summarised in tabular form:

Dalradian Succession: Blair Atholl to Loch Tummel.

Perthshire Quartzite Series	{	Carn Mairg Quartzite.
	{	Killiecrankie Schist.
	{	Schiehallion Quartzite.
Blair Atholl Series	{	Pale Group {
		{ Schiehallion Boulder Bed.
		{ White Limestone.
		{ Banded Group.
	{	Dark Group {
		{ Grey Limestone.
		{ Dark Schist.

3. *Rocks of the Banvie Burn Belt.*—Along Allt Bhaic, and occupying a varying width of country between the Struan Flags and the Loch Tay fault, there is another group of Dalradian rocks consisting essentially of limestone, schist, and quartzite, and referred to by Bailey (1925, pp. 684, 691) as the *Blair Atholl Series of the Banvie Burn Belt*. The stratigraphical position of the Banvie Series has always been a puzzle, but its problems lie outside the scope of the present work. It is hoped, however, to deal with it in a future paper.

4. *The Blair Atholl Slide.*—In order further to delimit the area dealt with in this paper it is necessary to refer to another aspect of Bailey's

work, this time concerning the structural relations of the Blair Atholl Series and the Perthshire Quartzite Series in the country to the south-east of Blair Atholl.

In the Killiecrankie district, and along the River Tummel east of Loch Tummel, the Perthshire Quartzite Series is represented by a very extensive outcrop of the Killiecrankie Schist, beautifully exposed in the famous Pass of Killiecrankie, in the River Tummel, and in the cliffs and steep slopes of the Tulach ridge opposite Killiecrankie village.

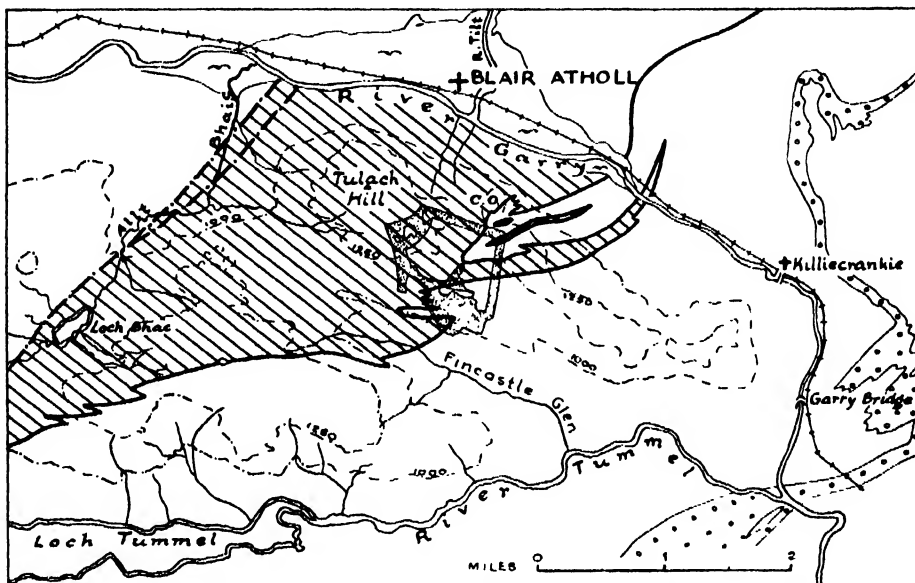


FIG. 1.—Index map. The lined area is that dealt with in the paper. The branched dot-and-dash line forming the north-west limit of the area is the Loch Tay fault; the heavy solid line on the south and south-east is the Blair Atholl Slide. The outcrop of the Carn Maig Quartzite is shown by small circles. C.O., about a mile east of Tulach Hill, is Creag Odhar. The densely dotted area south-east of Tulach Hill represents trees.

To the south-east the Killiecrankie Schist is followed by the Carn Maig Quartzite, and this in turn by the Ben Eagach Black Schist. To the north-west the Killiecrankie Schist is bounded by the Blair Atholl Grey Limestone; both the Schiehallion Quartzite and the Pale Group of Blair Atholl are absent for many miles along this junction. The great slide between the Killiecrankie Schist and the Grey Limestone has been called the *Northern Slide of the Tummel Belt* by Bailey (1925, p. 693); in the present paper it is referred to more briefly as the *Blair Atholl Slide*. It is represented by the heavy line in fig. 1. The highly sinuous nature of the outcrop, both here and in other parts of its course from Schiehallion

to within two miles of Braemar, points to much folding of the Blair Atholl Slide.

The area dealt with in this paper may now be defined as that lying between the River Garry on the north, the Loch Tay fault on the west, and the Blair Atholl Slide on the south and east. The small part of this area which lies south of Loch Bhac, however, will not be discussed.

5. The writer has remapped this district on the scale of six inches to the mile during the revision of Sheet 55 for the Geological Survey, and he is indebted to Dr E. B. Bailey for permission to publish this note and also for much valuable discussion in the field.

In Sheet 55 Grant Wilson indicated the existence of several outcrops of quartzite and quartz-schist on Tulach Hill and in the country a mile and a half to the south-west, as well as a large outcrop along Allt Bhaic. Bailey, on his small scale map (Plate, opposite p. 698, 1925), modified Wilson's mapping by discarding the outcrops of siliceous rocks on Tulach Hill and by reducing the area of the quartzite along Allt Bhaic. The Allt Bhaic quartzite was assigned by Bailey to an elongated outcrop of Schiehallion Quartzite between two branches of the Loch Tay fault.

The writer's interpretation is illustrated in the accompanying map (Pl.). He accepts from Bailey the Schiehallion Quartzite of Allt Bhaic, and follows Grant Wilson in mapping a considerable area of siliceous rocks (here interpreted as representing the Banded Group of the Blair Atholl Series) on the northern slopes of Tulach Hill.

STRATIGRAPHY.

Blair Atholl Series.

Dark Schist.—The Dark Schist is essentially a highly pelitic mica-schist with garnets; dark graphitic bands can usually be found in most exposures. It must be pointed out, however, that in many places on the south side of Tulach Hill the Dark Schist is not so conspicuously graphitic as the same rock in the section in the River Tilt below Old Bridge of Tilt at Blair Atholl. This Tilt section must be considered the best locality for the examination of the typical graphitic schists of the Dark Schist in the whole of the Blair Atholl region.

Several specimens of the rocks of the Dark Schist group have been sliced. A rather surprising feature of some of the graphitic mica-schists is their richness in epidote and zoisite. This applies particularly to the exposures on the summit of Tulach Hill. Some bands in these rocks are composed almost entirely of these two minerals.

In addition to the above rocks which contain graphite, there are others

associated with them that are free from this mineral. The rocks, as seen under the microscope, are essentially garnet-biotite-quartz-schists with bands of plagioclase and coarsely crystalline aggregates of chlorite. A specimen from the southern side of Tulach Hill contains abundant hornblende as well as plagioclase, biotite, chlorite, zoisite, quartz, and iron ore. The hornblende occurs as good green prisms usually associated with biotite, some of which has been formed from it. Both the hornblende and the biotite are frequently embedded in the large porphyroblasts of plagioclase. A hornblendic variety from the River Garry, west of Garryside Bridge, shows the hornblende largely replaced by chlorite and calcite.

Associated also with the graphitic schists along the Garry is a quartz-mica-schist containing pseudomorphs after staurolite and garnet. The staurolite is entirely or partly replaced by schimmer aggregates of white mica, and the garnet by chlorite with some scaly white mica.

Outcrop.—There are six important outcrops of the Blair Atholl Dark Schist in the Tulach Hill. (*a*) and (*b*) Beginning at the north, there are exposures in two of these along the River Garry about three-quarters of a mile west of Blair Atholl station. The more easterly outcrop is also exposed inland in a stream flowing to the Garry from the western slopes of Tulach Hill. (*c*) The next outcrop occurs farther up this stream and in the heather-covered slope to the east. It is shown on the map as ending eastward, and it is believed to belong to the same fold-core as the outcrop downstream to the north. These three outcrops lie on the north side of Tulach Hill. (*d*) Another belt of Dark Schist forms many of the smaller elevations on the summit of the hill, and is also exposed in the stream flowing down to the footbridge at Garryside and again in the River Garry itself at the bridge. (*e*) An extensive belt of Dark Schist outcrops on the lower ground on the south side of the main rise of the hill. This belt runs parallel with the summit outcrop and likewise bends northward round the eastern side of the hill. It is poorly exposed in a small stream to the east of the Garryside burn mentioned above. Towards the west it has an extensive outcrop, with several infolds of limestone, on the slopes and small hills between Tulach Hill and Loch Bhac. (*f*) The last-mentioned outcrop is bounded on the south and east by one of the most conspicuous limestone exposures found anywhere in the Central Highlands. In the region south-east of Tulach Hill this limestone has a double outcrop, with a large belt of Dark Schist between the two branches which are mapped as joining towards the west in the morainic area at the head of Allt Fearn, the stream which flows towards Fincastle House in Glen Fincastle.

Grey Limestone.—The present comparatively small area gives a striking display of the Grey Limestone. It is exposed in high cliff sections at Creag Odhar, one mile south-east of Blair Atholl, and in bare rock surfaces which can be followed for long distances on the south side of Tulach Hill. As far as Scotland is concerned the limestone exposures are probably second only to those of the Durness Limestone districts of the N.W. Highlands. There is also a fine quarry section in Grey Limestone a few yards to the west of Garryside bridge. This section shows disharmonic folding.

As in the case of the Dark Schist, the outcrops of the Grey Limestone are repeated several times and need not be enumerated here. As a rule, exposures are good, particularly above the drift line shown by dots on the accompanying map (Pl.). Attention, however, may be drawn to the fact that probably the best development of the Grey Limestone forms the southern and eastern limit of the Blair Atholl Series in the Tulach district, and that it is in contact with Killiecrankie Schist practically all the way from the Garry to the Loch Tay fault.

The Grey Limestone is remarkably uniform in character over the whole of the Blair Atholl region. For the most part it is a coarsely crystalline pale grey marble containing graphite, as has been pointed out in previous communications.

Banded Group.—The Banded Group is exposed in Allt Bhaic about two-thirds of a mile south of the farm of Balnansteuartach, and again in the east-and-west stretch of the small stream flowing from the north-western slopes of Tulach Hill to Allt Bhaic near Balnansteuartach. The rocks consist of thin-bedded quartzites, quartz-schists, and silvery mica-schists, associated with epidiorites at two places along the small tributary just mentioned. Elsewhere the outcrop is for the most part obscured by drift and heather, but three small exposures to the north and west of Tulach Hill provide additional evidence of its position between the two limestone outcrops shown on the map. In these exposures the Banded Group schists are in contact with the Grey Limestone. The exposures are easily found on the ground. They occur (1) where the Grey Limestone crosses a conspicuous fence, 600 yd. west of the summit of Tulach Hill, (2) 300 yd. east of the fence at locality (1), and (3) about 200 yd. west of north of the summit of Tulach Hill. In each of these exposures the Banded Group structurally underlies the Grey Limestone.

Another outcrop of Banded Group is shown on the map between the two branches of the Loch Tay fault, a mile west of Blair Atholl. The rocks are exposed at low water along the course of the River Garry. They are highly shattered and injected with much granitic material. Exposures

in this outcrop are poor in the present area, but good stream sections are found in the Banvie Burn, north-west of Blair Atholl and north of the Garry.

White Limestone.—There is only one exposure of White Limestone in the present area. It lies in Allt Bhaic on the southern side of the Banded Group mentioned at the beginning of the last section. The limestone is distinct from the Grey Limestone, with which it has previously been confused, in being essentially white in colour and rich in mica and tremolite. There is a great development of the White Limestone in a cliff section at this place. As we have pointed out, it is followed to the north by the Banded Series. To the south it is in contact with the Grey Limestone just less than 100 yards north of a small tributary (Allt Cosach) entering the Bhaic from the east.

An outcrop of White Limestone is shown on the map (Pl.) between the two branches of the Loch Tay fault. There are, however, no exposures in this locality.

Perthshire Quartzite Series.

Schiehallion Quartzite.—There are imposing cliff sections of highly broken fine-grained quartzite along Allt Bhaic between the two branches of the Loch Tay fault, half a mile south of Balnansteuartach. The outcrop extends for 400 yd. and, but for some thin calcareous and micaceous beds near the southern end of the outcrop, is composed entirely of quartzite.

STRUCTURE.

No attempt has been made by previous workers to unravel the details of the structure of the present district. It has been recognised by all that the rocks are "involved in the complex system of plications" which result in the beds being constantly repeated by folding and that they therefore exhibit extremely irregular outcrops (Grant Wilson, 1905, p. 32).

The question of the number of limestones with which we are concerned in the Tulach area is of prime importance in connection with the interpretation of the structure advanced in this paper.

It has already been pointed out that the Blair Atholl Series is now known to contain two important limestone horizons, the Grey Limestone and the White Limestone. Both of these are included in the Tulach area, but it is with the Grey Limestone that we are specially concerned now, for, as we have seen, there is only one exposure of White Limestone.

In the stretch of the River Tilt above and below Old Bridge of Tilt,

near Blair Atholl, a magnificent section of Grey Limestone is followed downstream by Dark Schist and upstream by various members of the Pale Group. The rocks are violently folded and with a little trouble the folds can be examined in the gorge immediately downstream from the bridge. In spite of the folding, however, it is comparatively easy to demonstrate that the Grey Limestone and Dark Schist are linked by interbanding along their junction. Within the belt mapped as Dark Schist there are numerous limestones. They are for the most part thin and distinctly dark in colour and dense in texture. Near the top of the

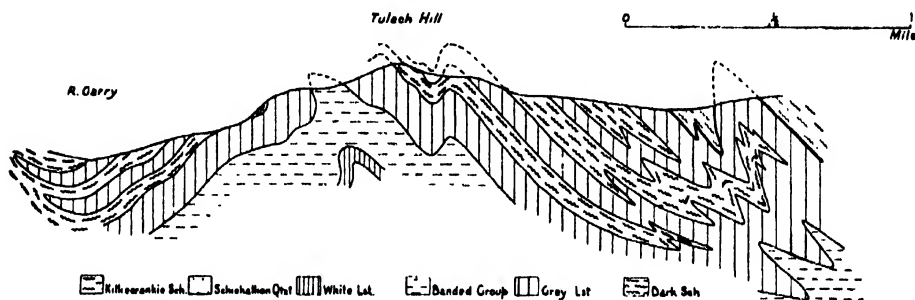


FIG. 2.--Generalised section across Tulach Hill.

Dark Schist, however, the interbedded limestones are of Grey Limestone type. Within the main outcrop of the Grey Limestone there are some bands of dark schist. In this part, therefore, it is clear that the Grey Limestone does not represent one thick limestone but consists of several limestones, how many it is impossible to say.

The interbanding of limestone and dark schist does not continue far into the main outcrop of the Grey Limestone, and in the writer's experience it is only possible to demonstrate the existence of thin bands of dark schist in this horizon in a perfectly clear river section such as that afforded by the Tilt. The writer is confident that elsewhere, as in the hill sections described in this paper, Grey Limestone outcrops separated by mappable Dark Schists must be interpreted as repetitions by recumbent folding of the main Grey Limestone and Dark Schist as illustrated in fig. 2. Examination of a large area outside the present district has convinced him of the truth of this interpretation.

Before considering the large-scale structure of the Tulach Hill district and its relation to surrounding areas it is necessary at this stage to review briefly the structure of the country to the north-east.

Bailey's structural succession in the Blair Atholl district may be summarised as follows.

The Perthshire Quartzite Series is disposed in two great recumbent

fold limbs called the Ben y Gloc and Tummel Limbs. These are separated by a fold core of Blair Atholl Series. North-east of Blair Atholl the Blair Atholl Series is seen to overlies structurally the Ben y Gloc Limb of Perthshire Quartzite Series, and it is assumed that the Blair Atholl core closes to the south-east.

These major tectonic elements are themselves considerably folded. For example, the Perthshire Quartzite Series of the Ben y Gloc Limb is folded into the Ben y Gloc and Meall Gruaim antiforms separated by

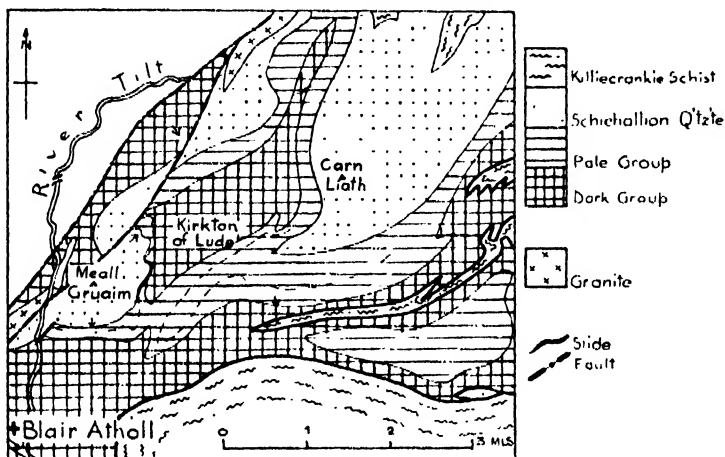


FIG. 3.—Geological map. Blair Atholl to Carn Liath (after Bailey and McCallicen).

the Kirkton of Lude synform of Blair Atholl Series. The Meall Gruaim antiform is followed to the north-west by a broken outcrop of Blair Atholl Series which we may call the Croftmore synform from the name of a farm overlooking the River Tilt west of Meall Gruaim.

The Meall Gruaim antiform is cut across and the parts displaced laterally by the south-eastern branch of the Loch Tay fault.

These structures are illustrated in figs. 3, 4.

Bailey's conclusions regarding the tectonics of the Ben y Gloc region are summarised because the writer hopes to demonstrate that the structure of Tulach Hill is a repetition of some at least of the major features just mentioned.

In the country north-east of Blair Atholl the folds pitch to the south and south-east as illustrated, for example, by the closing of the Ben y Gloc antiform at Carn Liath. Accordingly the writer believes that around Blair Atholl the Blair Atholl Series is underlain by the Perthshire Quartzite Series of the Ben y Gloc Limb.

The distribution of the rocks in Tulach Hill has already been described.

The most obvious major structure, which is self-evident in the field, is an antiform of Banded Group which rises up into the heart of the Dark Group from Allt Bhaic to beyond the summit of Tulach Hill. In the field it is possible to walk right round the close of this eastward pitching antiform. The meeting of the Grey Limestone of the southern limb with

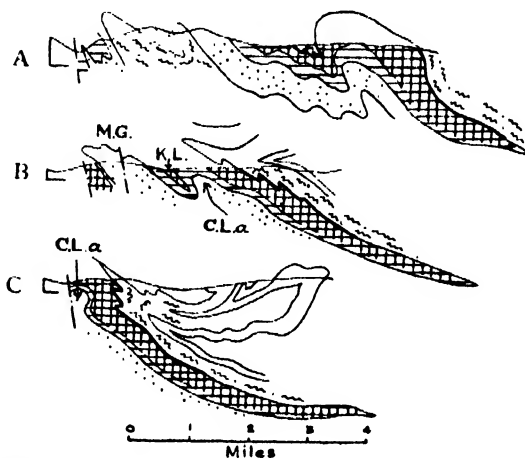


FIG. 4.—Sections from north-west (left-hand side) to south-east across the Blair Atholl district (after Bailey).

A, through Ben y Gloe; B, through Meall Gruaim; C, through Loch Bhac. In C only the part with which we are concerned is ornamented. Heavy lines, slides.

M.G., Meall Gruaim; K.L., Kirkton of Lude; C.L.a., Carn Liath (or Ben y Gloe) antiform.

that of the northern limb is only obscured for a few yards under turf. The Grey Limestone in its turn pitches eastward beneath Dark Schist.

On the high ground, the centre of the Tulach antiform is occupied by Banded Group, but, as previously mentioned, White Limestone occurs below the Banded Group in Allt Bhaic. In this part the southern limb of the antiform is replaced by a slide which brings the White Limestone into contact with the Grey Limestone.

We have now dealt with Tulach Hill and the country to the north and attempted a correlation of the structures in the two regions. It is apposite to consider certain other correlations made by Bailey and McCallien in 1937 between the Blair Atholl and Schiehallion districts on the two sides of the Loch Tay fault. Tulach Hill lies between these two regions, and in 1937 the details of its structure were unknown.

The great outcrop of Perthshire Quartzite Series, which forms the mountain mass of Schiehallion, is abruptly terminated to the east by a conspicuous slide which brings the Perthshire Quartzite into contact with the Dark Group of Blair Atholl. This is the Schiehallion Slide. The

Dark Group outcrop is bounded on its south and south-east side by another slide which introduces an extensive outcrop of Perthshire Quartzite Series. This outcrop has been correlated with the Tummel Belt on the east side of the Loch Tay fault, and the slide is therefore believed to be the continuation of the Blair Atholl Slide of the Blair Atholl-to-Braemar region.

The Ben y Gloe antiform is correlated with the broken antiform of Schiehallion in which the southern limb is replaced by the Schiehallion Slide. In the present paper the Tulach antiform is correlated with the Ben y Gloe antiform, and it has been pointed out that towards the west the Tulach antiform is broken by a slide. This slide is, therefore, probably the continuation of the Schiehallion Slide.

The conspicuous Balliemore antiform of the Schiehallion complex has been correlated with the Meall Gruaim antiform. The Allt Bhaic outcrop of Schiehallion Quartzite in the Tulach district is also correlated with the same structure.

SUMMARY.

The limestone and black schist outcrops of Tulach Hill are believed to lie in three recumbent folds which have been considerably refolded so that they now form a large antiform with axis running north-east-south-west along the northern side of the summit of Tulach Hill. The centre of the antiform is occupied by White Limestone in Allt Bhaic. In this stream the southern limb of the antiform is replaced by a slide which brings White Limestone against Grey Limestone. Elsewhere the centre of the antiform is occupied by Banded Group.

The Tulach Hill antiform is correlated with the better-known Ben y Gloe antiform of the region to the north-east which was originally described by Bailey.

Other structural correlations are made between the Ben y Gloe region, Tulach Hill, and Schiehallion.

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








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DESCRIPTION OF PLATE.

Geological Map: Tulach Hill, Blair Atholl, Perthshire.

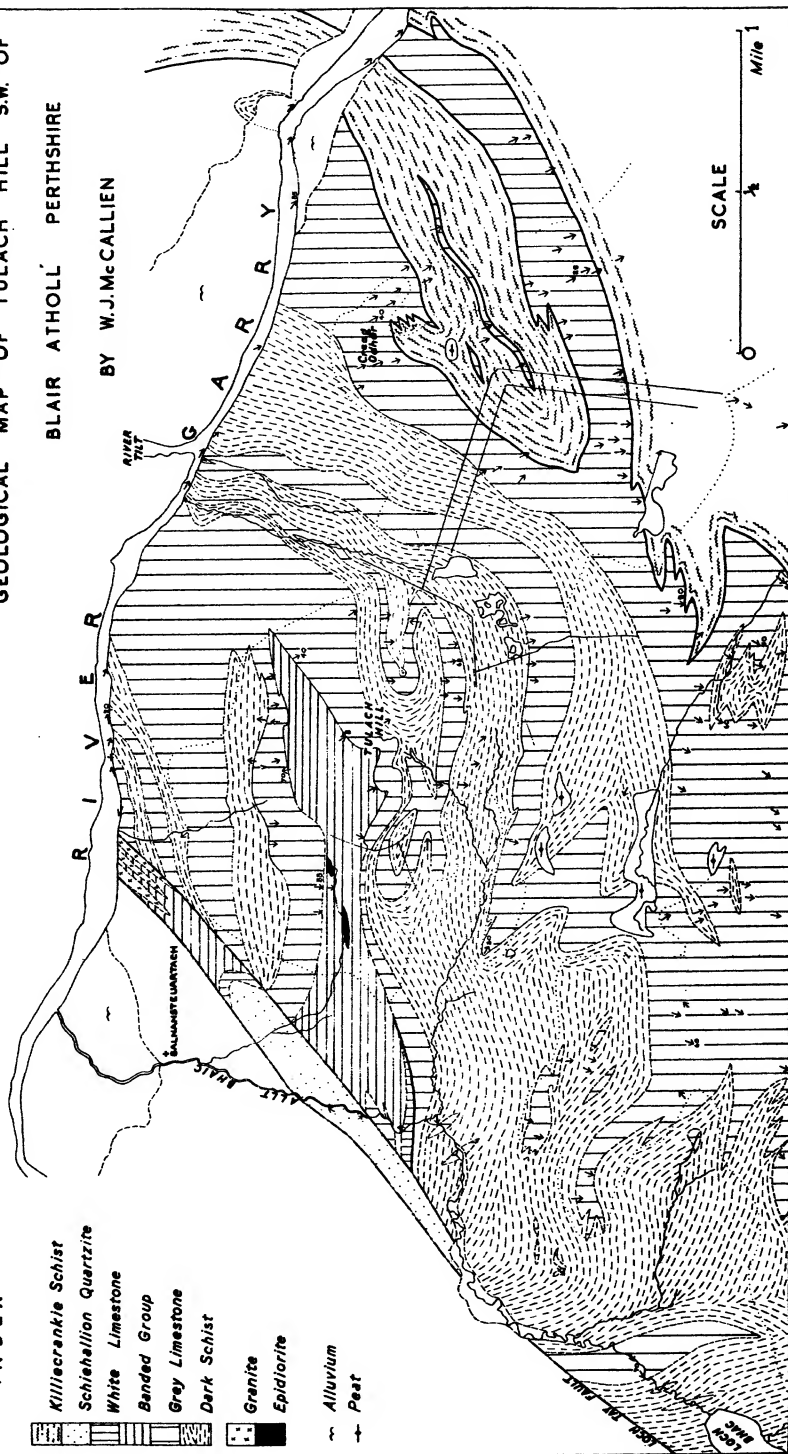
(Issued separately April 18, 1941.)

INDEX

-  Killiecrankie Schist
-  Schiehallion Quartzite
-  White Limestone
-  Banded Group
-  Grey Limestone
-  Dark Schist
-  Granite
-  Epidiorite
-  Alluvium
-  Peat

GEOLOGICAL MAP OF TULACH HILL S.W. OF BLAIR ATHOLL PERTHSHIRE

BY W.J.McCALLIEN



The dotted lines mark the boundaries of the drift-covered areas.

IV.—The Hydroid of the Medusa *Cosmetira pilosella* Forbes.

By W. J. Rees, Marine Biological Laboratory, Plymouth.

Communicated by Dr A. C. STEPHEN. (With Two Text-figures.)

(MS. received December 20, 1940. Read February 3, 1941.)

THE medusa *Cosmetira pilosella* Forbes is known, from the work of Miss M. J. Delap, to arise from a *Cuspidella* hydroid (Russell, 1936). Recently I found a small fertile colony of a *Cuspidella* on a dead bivalve shell dredged off Brigurd Buoy in the Firth of Clyde and this later liberated young medusæ in the laboratory. These were identified as

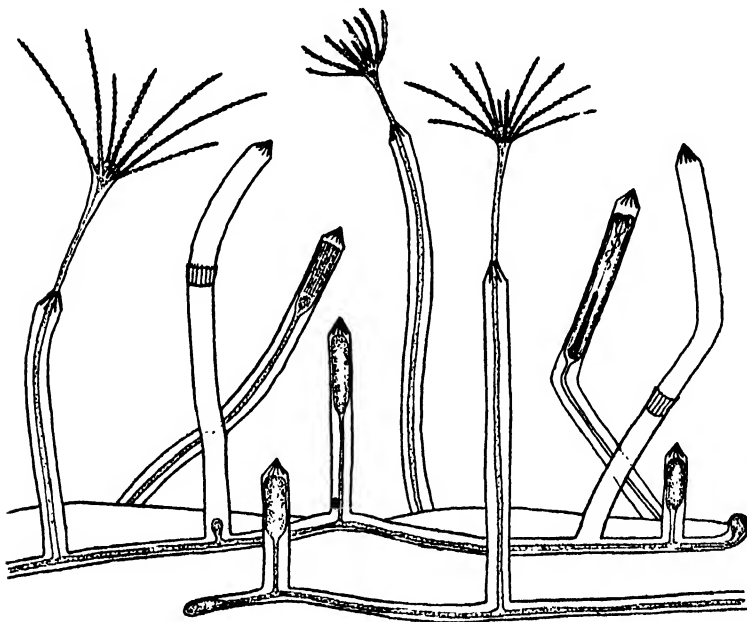


FIG. 1.—Hydroid of *Cosmetira pilosella* Forbes showing hydrothecæ and one gonotheca: Millport; 6. IV. 40.

Cosmetira pilosella, thus confirming that the hydroid of *C. pilosella* is a *Cuspidella*. There is no previous description of the hydroid or of the newly liberated medusa and so a description is given below.

The Hydroid (fig. 1).—The colony was found on April 5, 1940. It covered the greater part of one valve of the shell. There were a large

number of polyps, some connected by filiform creeping stolons. These stolons were firmly attached to the substratum and were very difficult to remove intact. They were smooth and the polyps arose directly from them without basal constrictions or annulations. The hydrothecæ varied considerably in length, with a maximum height (to the tip of the operculum) of 3.0 mm. They had a diameter of 0.14–0.15 mm.

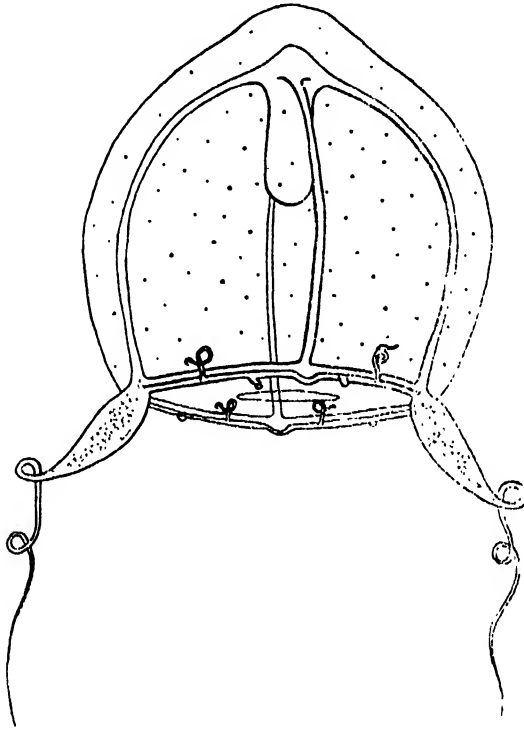


FIG. 2.—*Cosmetira pilosella* Forbes; newly liberated medusa: Millport, 6. IV. 40.

The hydrothecæ were nearly all long, smooth and tubular and only a few hydranths possessed growth rings. There was a distinct conical operculum with 8–12 segments; the usual number being ten. The height of the operculum was 0.14–0.15 mm. The hydranths were extensile and could extend beyond the operculum to at least half the height of the hydrotheca. There were eight to ten filiform tentacles with irregularly distributed nematocysts. The tentacles were situated in a single whorl around the base of a bluntly conical hypostome.

When found the colony was fertile and two medusæ were liberated in the laboratory on April 6, 1940. The gonothecæ did not differ in

size or in form from the hydrothecæ. Each contained only one medusa (fig. 1).

The Medusa.—The newly liberated medusa was of a deep bell-shape, 0.8 mm. in height (fig. 2). There was a slight apical projection and the umbrella was of uniform thickness and studded with exumbrellar nematocysts. The velum was well developed. The stomach was short, tubular, and the mouth simple, without lips. The four radial canals were well developed and united at the margin by a circular canal. There were two, opposite, well-developed perradial tentacles, each with a large, prominent spindle-shaped bulb. On the other two perradii the tentacle bulbs were very rudimentary. Situated adradially on the margin there were eight marginal cirri; four of these were well developed and the other four were less well developed and often rudimentary. There were no eyespots or marginal vesicles present. The base of the stomach and the tentacle bulbs were brownish in colour.

The hydroid is here also recorded from Plymouth. The hydroid was growing on a broken tea-cup dredged on the Eddystone Grounds on March 25, 1936. It was isolated in a finger-bowl and later, on March 28, I found a young medusa identical with the above in the bowl. I did not see the medusa before liberation and so I was not quite certain that the medusa had come from the *Cuspidella*. The observations made at Millport confirm this, and it is reasonable to assume that the hydroid from the Eddystone Grounds was this species.

DISCUSSION.

Of the three British species of *Cuspidella*, *C. costata* Hincks, *C. humilis* Hincks, and *C. grandis* Hincks, the hydroid of *Cosmetira pilosella* approaches nearest to *C. grandis* in the form of the hydrotheca. The figures given by Hincks (1868) and Hartlaub (1897) indicate that it may be identical with the present species. Browne (1907) and Russell (1936) have tentatively suggested that *C. costata* and *C. humilis* are the same species and there is some evidence to indicate that this species (i.e. *C. costata* and *C. humilis*) may be identical with the hydroid of *Laodicea undulata* described by Russell. It is also likely that *C. grandis* is identical with the hydroid of *Cosmetira pilosella*. However, as Russell has pointed out, final conclusions regarding the various species of *Cuspidella* cannot be reached until the life-history of all medusæ possessing *Cuspidella* hydroids have been carefully worked out.

These observations were made while I was holding a grant from the Royal Society.

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(Issued separately April 18, 1941.)

V.—On *Cardiopteridium*, a Genus of Fossil Plants of Lower Carboniferous Age, with Special Reference to Scottish Specimens. By **John Walton, M.A., D.Sc.**, Regius Professor of Botany in the University of Glasgow. (With One Plate and Three Text-figures.)

(MS. received January 28, 1941. Read May 5, 1941.)

INTRODUCTION.

AMONG the genera of fern-like plants of Palæozoic Age there are a number which have orbicular or reniform leaflets. Detached leaflets of this form are frequently found, and it is a matter of difficulty to determine to which of several genera such fragments belong.

Under the name *Cyclopteris* Brongniart (1828, p. 215) described some orbicular leaflets found in Upper Carboniferous strata, and later investigators have shown that most of these leaflets were the basal or stipular pinnules (aphlebiæ) of the frond of the Pteridosperm *Neuropteris*. They were sessile on the rachis and had broad bases of attachment, while the shape of the pinnules on the upper parts of the frond was quite different. The Scottish fossils which will be described in this memoir are also orbicular leaflets and have been named *Cyclopteris* by some authors. There is evidence, however, that they were pedicellate and were not the aphlebiæ of the frond which bore them.

The Devonian, Carboniferous, and Permian genus *Psymphyllum* Schimper (see Seward, 1919, p. 79) has large cuncate leaves which bear some resemblance to imperfect or torn specimens of *Cyclopteris*. Most forms of *Psymphyllum* are clearly lobed and may be thereby distinguished from *Cyclopteris*.

In *Cardiopteris* Schimper the frond was pinnate, the straight rachis bearing on each side a row of broadly sessile oval or orbicular leaflets. *Cardiopteris* is of Lower Carboniferous Age and has a wide geographical distribution (Seward, 1931, p. 191), but no specimens have been found in Britain. British fossils which have been named *Cardiopteris* are probably all referable to the genera *Cardiopteridium* or *Cyclopteris*. In the Upper Devonian genus *Archæopteris* and in the Lower Carboniferous *Rhacopteris* the form of the frond and the shape of the leaflets are

sufficiently distinct in character for us to recognise them. In the Lower Carboniferous genus *Adiantites* the leaflets tend to be cuneate rather than orbicular, but are similar in venation to the Scottish specimens described below and the form of the frond was probably similar.

The earliest description of a plant which bears a close resemblance to the Scottish specimens was given by Eichwald (1860, p. 64). He described a plant from the Ural Mountains near Sverdlovsk in Siberia, to which he gave the name *Cyclopteris nanus*. The rachises were slender and branched and the leaflets were mostly orbicular. Some of the leaflets appear to have been cuneate, but like several of the Scottish examples this may have been due to tearing. Some of the Scottish leaflets resemble those figured by Eichwald very closely, and Kidston, judging from his unpublished notes, regarded them as belonging to the same species. Nathorst (1914, p. 16) described some similar remains with smaller leaflets from the Culm of Spitsbergen under the name *Cardiopteridium spetsbergense*, recognising that the frond which bore these leaflets differed in construction from the true *Cardiopteris* and fronds which bore *Cardiopteris*-type of *aphlebiæ*. In 1932 Zimmermann gave an illustrated account of a plant closely similar to Nathorst's which he named *Cardiopteridium Waldenburgense*. He considered it to be distinct from *C. spetsbergense* and *Cyclopteris nanus*, but the distinctions are difficult to define and I do not think that these three sets of fossils are specifically distinct.

Among the Scottish specimens the larger leaflets resemble those of *Cyclopteris nanus* Eichwald, while the smaller specimens may be matched with those of *Cardiopteridium spetsbergense* and *C. Waldenburgense*. There is no clearly defined difference between the leaflets belonging to these three species and the Scottish specimens; they furnish an almost continuous series as regards size. I therefore consider it expedient to include them all in the same species *Cardiopteridium nanum* Eich. sp. and to recognise provisionally two formæ, f. *spetsbergensis* for the small leaflets and f. *typica* for the larger leaflets respectively. There is, however, no sharp line of demarcation between these two forms, and it is possible that they merely represent the extremes of a single species.

SCOTTISH SPECIMENS OF CARDIOPTERIDIUM.

Considerable numbers of detached orbicular or reniform leaflets have been found in the Lower Carboniferous Rocks of the Firth of Forth area. Most have been found in the Oil Shale Group of the Calciferous Sandstone Series, but some have been found in the Carboniferous Limestone Series.

Up to the present these Scottish fossils have been named by authors *Cardiopteris* or *Cyclopteris*. Some of the smallest are narrow elliptical, and the largest are usually reniform. The intermediate sizes approximate to orbicular in shape. The smaller examples have been found mostly in the Oil Shale Group in the neighbourhood of Edinburgh, where they occur in great abundance in some strata. The larger sizes are mostly from exposures of the Calciferous Sandstone Series on the shores of Fife and Haddington. Examples of the larger and smaller types have been found together at Pittenweem in Fife.

The main object of this memoir is to describe the rare examples in which these leaflets are attached to fragments of rachis and which give information about the form of the frond. Most of these specimens are in the Kidston Collection in the Geological Survey and Museum in London, while the remainder are in the Geological Survey Collection in Edinburgh and in the Hunterian Collection of Fossil Plants in the Department of Botany in the University of Glasgow.

Cardiopteridium nanum f. *spetsbergensis* n.f.

Pl., figs. 1, 2.

Cardiopteris sp. Stur., 1877, p. 182; pl. 11, fig. 6.

Neuropteris sp. Kidston, 1882, p. 541.

Cardiopteris polymorpha Kidston, 1894, p. 247.

„ „ Kidston, 1903, pp. 749, 819.

Adiantites spetsbergense, Nathorst, 1910, p. 329.

Cardiopteridium spetsbergense Nathorst, 1914, p. 16; pl. 1, figs. 6-15; pl. 8, figs. 5, 6.

Cardiopteris sp. Carpentier, 1924, p. 129; pl. 5, figs. 1-7.

Cardiopteridium Waldenburgense Zimmermann, 1932, p. 219; pl. 23, figs. 1-8; pl. 24, figs. 1-6.

Cardiopteris Crookall, 1932, p. 91.

Two examples of this form are shown in Pl., figs. 1, 2. The frond was evidently not simply pinnate, and the information given by these specimens is consistent with the suggestion made by Zimmermann (1932, p. 225) that the frond was, as regards the branching of its rachis, similar to that of *Adiantites oblongifolius*. The slender attachments of the leaflets to the rachis are visible in the photographs. The leaflets at the extremities of the rachises are frequently narrowed at the base, but those farther back have a circular or even reniform shape. The veins of the leaflets converge until they apparently fuse together and enter the pedicel of the leaflet. Large numbers of detached leaflets have been examined and they all show, if sufficiently complete, this convergence of the veins to a very narrow base.

A few simple hairs are present on the rachises of the two specimens illustrated here (figs. 1, 2); they are, however, not so abundant as those shown in Zimmermann's illustrations.

Cardiopteridium nanum Eich. sp. f. *typica*; n.f.

Text-figs. 1, 2; Pl., figs. 3, 4, 5.

Cyclopteris nana Eichwald, 1860, p. 64; pl. 1a, fig. 7.

Anemites nanus Schmalhausen, 1883, p. 6; pl. 1, figs. 6-9.

The specimen shown in Pl., fig. 3, can be matched exactly as regards form and venation with some illustrated by Zimmermann, but

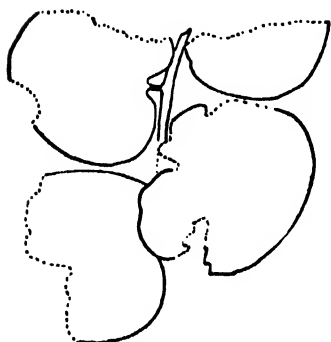


FIG. 1.—Outline sketch of part of a frond of *Cardiopteridium nanum* f. *typica*. $\frac{1}{2}$ nat. size. Shore at Archerfield, Dirleton, Haddingtonshire. Calciferous Sandstone Series. Kidston Collection, no. 5278, Geol. Surv. London.

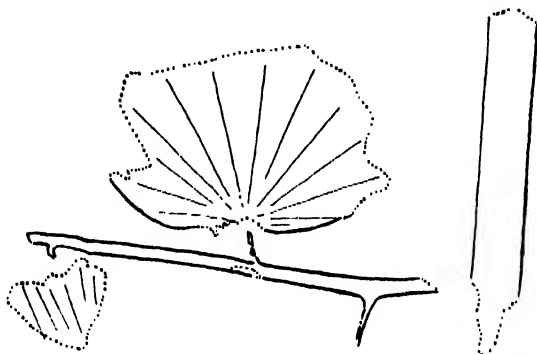


FIG. 2.—Large leaflet and small portion of a rachis of *Cardiopteridium nanum* f. *typica*. $\frac{1}{2}$ nat. size. The lines drawn on the lamina indicate the trend of the veins which are too numerous to be represented accurately. Anstruther Harbour, Fife. Calciferous Sandstone Series. Hunterian Mus., Univ. of Glasgow, no. Pb. 2218.

is considerably larger. Fig. 4 shows a leaflet of this size attached to a rachis which shows longitudinal striation similar to that described in *C. nanum*, *C. spetsbergense*, and *C. Waldenburgense*. These leaflets are reniform in shape, a feature also observed in some of the larger examples of f. *spetsbergensis*. In text-fig. 1 leaflets of similar size and form are shown with part of the rachis that bore them. The rachises shown in text-fig. 2 are longitudinally striated. The leaflet shown in this figure is about 80 mm. wide.

In the accompanying Table, *A* gives the widths of some of the best preserved leaflets found in Scotland arranged in a series with the forms previously described. In column *B* are given estimates of the number of veins per centimetre at the distal edge of the leaflet opposite its region of attachment.

It appears that the numbers in column *B* (2, 4, 8), based on measurements from the drawings by Nathorst and Schmalhausen, are smaller

than might be expected. This is possibly due to the difficulty the draughtsman had in representing the full number of very small veins. There is evidently an inverse relation between the size of the leaf and the vein density. The fairly uniform decrease in vein density with increase in size of leaflet supports the view that it is impossible to separate these forms into distinct species. It is, however, noticeable that the two forms recognised here are usually not found together in the same beds. This might be due to physical causes, for the smaller leaflets are usually found in fine sediments, while the larger ones are found in coarser sediments.

TABLE TO SHOW THE RELATION BETWEEN WIDTH OF LEAFLET AND
APPROXIMATE NUMBER OF VEINS PER CM. OF MARGIN.

	<i>A.</i> Width of Leaflet.	<i>B.</i> Veins per cm.
<i>Cardiopteridium nanum</i> Eichwald sp.		
1. Specimen shown in Pl., fig. 2	9	38
2. " <i>C. spetsbergense</i> ," Nathorst, 1914, pl. I, fig. 14	12	28
3. Specimen shown in Pl., fig. 1	15	36
4. " <i>Cardiopterus</i> sp.," Stur., 1877, pl. II, fig. 6	15	28
5. " " Carpentier, 1924, pl. 5, fig. 2	15	30
6. <i>C. waldenburgense</i> , Zimmermann, 1932, pl. 24, fig. 6	15	36
7. Specimen Pb. 2222, Hunterian Museum	17	32
8. " <i>Aneimites nanus</i> ," Schmalhausen. 1883, pl. I, fig. 7	20	26
9. Specimen Pb. 2223, Hunterian Museum	22	32
10. " Pb. 2225, " "	25	30
11. Specimen shown in Pl., fig. 3	30	30
12. " <i>Aneimites nanus</i> ," Schmalhausen, 1883, pl. I, fig. 9	40	23
13. Specimen Pb. 2221, Hunterian Museum	45	30
14. Specimen shown in Pl., fig. 4	50	28
15. " " , text-fig. 1	52	26
16. " " , Pl., fig. 5	60	22
17. " " , text-fig. 2	80	20
18. " " , " 3 and Pl., fig. 6	about 170	37
<i>Saportæa nervosa</i> Halle, 1927, pl. 55, fig. 3	about 110	40

Cardiopteridium cf. *nanum* f. *typica*.

Cf. *Noeggerathia dispar* Dawson, 1866, p. 153; pl. 13, fig. 91.

The specimen of which an outline drawing is given half natural size in text-fig. 3 was found by Mr W. Manson of the Scottish Geological Survey in beds of the Calcareous Sandstone Series on the shore at Temple, Lower Largo, Fife. Parts of four leaflets are shown, three of which were undoubtedly attached to the rachis, which is seen in the centre of the specimen. The leaflets were evidently attached alternately on opposite sides of the rachis, which is striated longitudinally. The leaflets had short pedicels and, judging from what is preserved of the lower one on the left, must have been flabellate or possibly reniform in shape. The vascular supply from the pedicel, unlike that in the other specimens of *Cardiopteridium*, divides into two main divisions which

extend along the margins on each side and give rise to the fine dichotomising veins (Pl., fig. 6), which spread through the lamina to the distal margin of the leaflet. There are about 37 veins per centimetre, an appreciably greater number than that found in the other large specimens. It thus differs from the other specimens in the katadromic arrangement of the veins and in the greater vein density. The fact that the leaflets are larger and the rachis stouter than in the other specimens suggest that perhaps it is part of the main rachis or petiole of a frond. This may be an explanation of the difference in shape of the leaflets, for in many

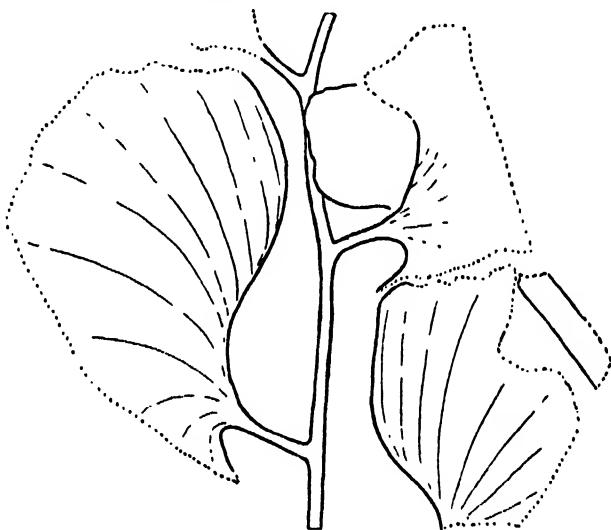


FIG. 3.—*Cardiopteridium* cf. *nanum* f. *typica*. Part of a frond showing portions of four leaflets. $\frac{1}{4}$ nat. size. Part of the leaflet on the left is shown nat. size in Pl., fig. 6. Shore at Temple, Lower Largo, Fife. Calciferous Sandstone Series. Hunterian Mus., Univ. of Glasgow, no. Pb. 2215.

Palæozoic fronds the leaflets on the petiole differ in form from those on the branches of the rachis.

A similar katadromic arrangement of the veins is found in some of the living ferns (Bower, 1923, p. 88), in some leaves of *Ginkgo*, and in the Permian genus *Saportea*.

Sir Albert Seward has pointed out to me the resemblance between this specimen and *Saportea*, a genus which has so far been found only in the Permian of Virginia (Fontaine and White, 1880) and China (Halle, 1927, p. 194). The leaves of *Saportea* range from 7 cm. to 20 cm. in width; they are reniform or flabellate and have the same katadromic venation. The petiole, however, is much longer, attaining a length of 5 cm. Halle places *Saportea* in the Ginkgoales.

While this Fife specimen shows points of resemblance to *Saportea*, its

occurrence at a horizon in the Lower Carboniferous at which in nearby localities *Cardiopteridium* is common and its resemblance in habit to the latter genus throws considerable doubt on a relationship to *Saportæa*. No plants resembling *Cardiopteridium* have been found in the Permian of Virginia or China.

From an examination of the available records and specimens it is clear that *Cardiopteridium* as far as is known occurs exclusively in Lower Carboniferous rocks. In Britain it is recorded from a considerable number of localities in the Lothians and in Fife. It is also found in Dumfriesshire and Northumberland near the border. Carpentier (1924, p. 125) figures specimens from the Culm Supérieur of Mayenne in France. Zimmermann records it from the Lower Carboniferous of Silesia and Nathorst from the Culm of Spitsbergen. The earliest record is Eichwald's from the Lower Carboniferous on the eastern slopes of the Ural Mountains near Sverdlovsk in Siberia. No evidence has been found of the presence of *Cardiopteris* in Britain. All records of this genus in Britain are probably incorrect; the specimens on which they are based belong to the genus *Cardiopteridium*.

I acknowledge with thanks the permission granted by the Geological Survey authorities to figure and describe specimens from their collections. I have also to thank Mr William C. Anderson and Mr W. Manson for generously putting at my disposal important specimens which they have collected on the Fife coast.

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DESCRIPTION OF PLATE.

Cardiopteridium nanum f. *spetsbergense*.

Fig. 1. Part of a frond bearing about fourteen leaflets. Preserved in mudstone. $\times 0.91$. Hailes Quarry, near Kingsknowe, Midlothian. Wardie Shales, Oil Shale Group, Calciferous Sandstone Series, Lower Carboniferous. Geol. Surv. Edin. T. 43813.

Fig. 2. Part of a frond showing five leaflets in oil shale. $\times 2.75$. Same locality and horizon as for fig. 1. Univ. Glasgow, Hunterian Coll., no. Pb. 2216.

Cardiopteridium nanum f. *typica*.

Fig. 3. Parts of two leaflets. $\times 0.91$. Kenly Den, Strathvithie, Fife. Calciferous Sandstone Series. Geol. Surv. London, Kidston Coll., no. 879.

Fig. 4. Rachis bearing one leaflet on pedicel. $\times 0.91$. Shore at Archerfield, Dirleton, Haddington. Calciferous Sandstone Series. Kidston Coll., no. 5279.

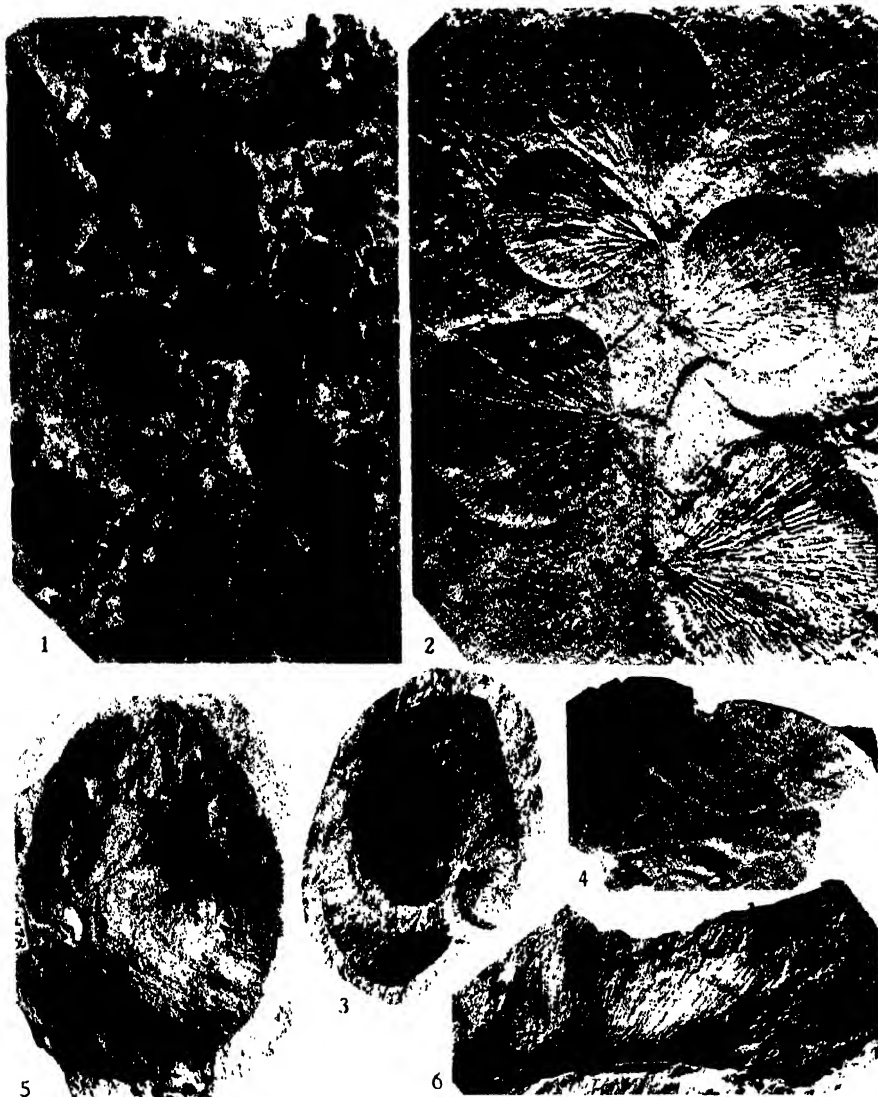
Fig. 5. Single leaflet. $\times 0.91$. Same locality and horizon as for fig. 3.

Cardiopteridium cf. *nanum* f. *typica*.

Fig. 6. Small part of the proximal edge of the left-hand leaflet of the specimen shown in text-fig. 3 to show the venation. $\times 0.91$. Shore at Temple, Lower Largo, Fife. Calciferous Sandstone Series. Coll. by W. Manson, Esq. Hunterian Museum, Univ. of Glasgow, no. Pb. 2215.

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(Issued separately July 25, 1941.)



Zineo-Clototype Co., Edinburgh.

SCOTTISH SPECIMENS OF *CARDIOPTERIDIUM*

VI.—Mutation and Lethal Effects of Ultra-Violet Irradiation on *Drosophila*. By **K. Mackenzie**, Ph.D., Institute of Animal Genetics, University of Edinburgh. *Communicated by Dr P. C. KOLLER.* (With Two Text-figures.)

(MS. received December 12, 1940. Revised MS. received February 2, 1941.
Read May 5, 1941.)

IN a recent paper (Mackenzie and Muller, 1940) a brief account was given of certain methods employed to obtain a favourable mutation rate in male adult *Drosophila*. These methods were largely empirical, and a detailed account of their development, together with a discussion of their significance, seemed rather beyond the scope of the paper, which dealt principally with the possible chromosome rearrangements which could be brought about by ultra-violet light. Hence only the average of a large number of experiments was given. In this paper it is proposed to give a fuller account of the influence of ultra-violet rays on *Drosophila*, and to compare the somatic effects, which are directly lethal, with the spermatic effects, which occur in the surviving insects and are observed as mutations in the offspring.

Previous workers on this subject, using somewhat simpler methods, have confined themselves almost exclusively to the genetical results. Altenburg (1930, 1931, 1934) used chiefly unfiltered radiation of low intensity from an air-cooled quartz mercury lamp (*i.e.* the flies were exposed at 3 m. distance for periods up to 44 hours). The mutation rate was small, amounting to 34 mutations in 11,500 cultures; the dosage was not ascertained by physical methods, but by the effect upon the flies. In conversation with Professor Altenburg, he stated that the relation between the direct lethal effect and the genetic effect was well known to him, and that the dose given was near the margin of lethality. Owing to the technical difficulties at that time existing both in ultra-violet measurement and genetical analysis, the definite findings of Altenburg represent a triumph of patience and perseverance.

Reuss (1935) used filtered radiation of wave-length 400–280 m μ by the aid of "Uviol" glasses, and, by fixing and orienting the flies on a pad of cotton-wool, was able to increase the mutation rate, up to 6.5 ± 1.15 per cent., with an average of 4.14 ± 0.36 per cent. A control

experiment with ordinary glass gave a mutation rate of 0.12 ± 0.09 per cent. By the use of various filters he showed that $313 \text{ m}\mu$ is the upper limit of ultra-violet waves producing mutations. The dosage was given in terms of time and distance from the ultra-violet source (usually 20–30 minutes at 25–40 cm.). Though not directly stated, it may be assumed that the dose was adjusted to a level a little below that at which the flies would be killed by the incident radiation.

Eloff and Bosazza (1938) irradiated *Drosophila* pupæ with various wave-lengths from $365\text{--}225 \text{ m}\mu$, using layers of chitin (flies' wings) as a filtering medium. Somatic mutations, observed as wing abnormalities, varied from 0 per cent. with $365 \text{ m}\mu$ to 60 per cent. with $225 \text{ m}\mu$.

McAulay and Taylor (1939) observed lethal and quasi-lethal effects of monochromatic ultra-violet wave-lengths between 254 and $365 \text{ m}\mu$ on *Drosophila* larvæ (among other biological material). The dose required for ultimate death was some 2×10^7 ergs/cm.² at $254 \text{ m}\mu$ and increased to about 5×10^7 ergs at $313 \text{ m}\mu$, and to 30×10^7 ergs at 365 . Ten times this dosage caused almost immediate death. It is somewhat difficult to make a comparison of the sensitivity of the larva with that of the adult, as they may differ profoundly in their morphology and have much less chitin, which filters off the ultra-violet. The lethal effects in larvæ were apparently not due to surface heating by absorption, as they were immersed in water during irradiation.

EXPERIMENTAL.

A water-cooled quartz capillary mercury arc of the type described by Daniels *et al.* (1932) was used as a source of ultra-violet light. It was enclosed in a stout metal case with a window 5 cm. square made of Schott und Genossen U.G.2 glass 1 mm. thick. The water stream at high pressure passed over the quartz capillary and impinged directly on the window, keeping its inner surface at a temperature little above that of the water. The arc, which was confined to a volume of $25\text{--}50 \text{ mm}^3$ at a pressure of one to two atmospheres, dissipated about 500–600 watts at 250–300 volts. Under these conditions the quartz tube had a useful life of 20–50 hours. It usually failed owing to internal stresses, or became too opaque to the shorter wave-lengths by internal fusion or external deposits on the capillary. If the water-supply fell off or ceased it disintegrated immediately. The radiation from the lamp, especially when new, was so great that the filter, which absorbed more than 90 per cent. of the total energy, was subject to great strain. Its temperature on the outer surface often exceeded 70° , while its internal surface at 1 mm. distance was kept at 20° or below, and it frequently cracked across.

Regulation of the current by resistances in the external circuit enabled a new lamp to be run at a somewhat lower voltage, which was raised as the lamp got older. The properties of the pressure capillary arc are discussed in detail by Daniels *et al.* Owing to the source being practically a line and efficiently cooled, it was possible to work at short distances, 5 cm. or over, and obtain a very intense and constant radiation between the λ 400 $m\mu$ and 280 $m\mu$. The approximate distribution for an average lamp by a thermopile and monochromator is:

TABLE I.

Region	400-340	340-320	320-300	300-290	below 290
Principal line	365	334	313,302	297	280
Energy in 10^8 ergs/sec./cm. ² at 10 cm.	125	22	8.8	5.4	<0.1
Relative intensity	14	2.5	1.0	0.6	<0.01
Relative intensity with additional Ag filter (<i>vide supra</i>).	0.8	0.3	0.87	0.9	<0.01

By means of an accessory filter, consisting of a thin film of silver chemically deposited on a quartz plate, it was possible to restrict the spectral region still further, using a band from 320 to 290 $m\mu$, together with a small proportion of the longer wave-lengths. This was found to be equally effective in producing mutations, while reducing the total incident radiation by a factor of from 5 to 20. Hence it is possible, in agreement with the experiments of Reuss, to consider the strong lines at 365 and 334 $m\mu$ (in the mercury vapour spectrum) as biologically ineffective (Mackenzie and Muller, 1940).

Method of Measurement.—A Cambridge thermopile, with a slit of 1.25 mm., was used, giving a current of about 1 microampere for an incident radiation of about 1×10^8 ergs/cm.²/sec.; it was coupled to a Tinsley D'Arsonval galvanometer giving a deflection of about 300 mm. per microampere at 1 m. The zero remained constant to about 5 mm., provided the thermopile was exposed for not more than 5 minutes in every hour to check the radiation from the lamp. The output seldom varied by 5 per cent. during a single irradiation, which never took more than 60 minutes. The irradiation value was taken as the average of not less than three readings during the irradiation period.

The thermopile-galvanometer combination was calibrated by means of a secondary standard, a carbon lamp from the U.S. Bureau of Standards operated at the current and voltage specified. It is probable that the values given fall within ± 5 per cent. of the absolute value, though the standard was almost entirely emitting on wave-lengths about 500 $m\mu$,

while the energy measured in the experimental work was below 400 $m\mu$, and usually below 330 $m\mu$. The thermopile was carefully blackened before use, and maintained under as constant conditions as possible.

Treatment of the Flies.—The young adult male *Drosophila* were separated from the parent cultures, and while still etherised were placed on a thick pad of cotton-wool, about 5 cm. square, backed with a sheet of metal of similar size. They were carefully oriented so that they lay on their backs, and were then covered with a sheet of clear quartz, 5 cm. square, care being taken not to displace the insects. By means of stout rubber bands near the edges, the front and rear plates were firmly bound together under a degree of compression which flattened the insects to an extent sufficient to prevent them from moving, but did not seriously incommode them. Reuss recommends that the head and thorax should be protected by spotting with Indian ink, but this was not found to produce any difference in the results. The insects thus confined, varying from 50 to 70 in number, were then exposed at a distance of 5–20 cm. from the ultra-violet source. The thermopile placed at 20 cm. was used to give readings of the intensity at the beginning of the run, and every 5–10 minutes thereafter, depending on the total time required. After the allotted exposure had been given, the flies were examined to make sure their orientation was still correct, etherised, and transferred to culture bottles or vials.

Effect of Varying the Dose.—Doses varying from 60 per cent. to 150 per cent. of the standard dose were employed. The test was performed on the same number of flies in each case, being 200 in all, and not more than 70 were treated at any one time, this being the largest number which could be conveniently treated under the standard conditions. The total of 200 was made up of at least three groups of flies, and the dosage was therefore the average of three trials and was less likely to be subject to serious error. The males were placed in separate vials, with two virgin females of the stock to which they were to be crossed. These vials were examined at 24 hours, 72 hours, and 14 days after making up. On the first two occasions the vials in which the males were no longer alive were separated from the remainder; on the third occasion all flies in the vials which had given rise to offspring were inspected. The flies were etherised, the number counted, and they were examined microscopically for irregularities suggestive of mutations. (Full details of the genetical technique are given in the paper by Mackenzie and Muller, 1940.)

It was noted almost from the commencement of the experiments that there was a close parallelism between the number of flies killed by a given

dose of ultra-violet irradiation and the genetic effectiveness of the treatment. Indeed, the original method of obtaining satisfactory genetical results was to divide a group of male flies into three or four parts, giving a dose increasing successively by steps of 50 per cent. These were then cultured with virgin females, and the highest dose at which any fertile culture could be obtained was used for genetic tests. This enabled the maximum tolerance dose to be estimated roughly. When this had been ascertained, the relative doses given to the various groups were brought much closer together. Increments of 25 per cent., and subsequently 10 per cent., were employed, and as this last was about the limit of accuracy in working, this was finally adopted as standard. The group of flies was usually divided into three lots, and these were given doses of 100, 110, and 120 per cent. of the standard dose employed in any particular experiment.

Effectiveness of Emission.—It was found that the total radiation from the lamp, when screened by the Uviol filter, could vary over limits of 300 per cent. without noticeably affecting the genetic and lethal response, if the time of exposure was adjusted to compensate for the greater or smaller emission in the effective region (see Table II).

TABLE II.—EFFECT OF VARIATION OF U.V. EMISSION ON THE LETHAL AND MUTATION RATES.

Description of Lamp.	1 High Pressure.	2 High Pressure.	3 Moderate Pressure.	4 Low Pressure.
Age	About 1 hr.	About 40 hrs. (same as 1)	5 hrs.	3 hrs.
Voltage	360	320	240	180
Current (amps.)	1.2	1.3	1.8	2.8
Watts	420	420	430	500
Emission in 10^8 ergs/sec./cm. ² at 10 cm.—				
(a) 400–280 $m\mu$	245	80	130	120
(b) 330–280 $m\mu$	53	12	22	16
Total flies exposed	130	220	200	200
Time of exposure (at 10 cm.)— mins.	6	27	14	18
Dose $\times 10^7$ ergs/cm. ² —				
(a) 400–280 $m\mu$	8.8	12.96	10.9	12.96
(b) 330–280 $m\mu$	1.91	1.94	1.85	1.73
Mutation rate per cent. . .	4.2	3.8	4.4	3.9
Lethal effect per cent. . . .	26	40	37	41

This shows that the wave-lengths principally effective in producing mutations, with the filters and conditions used, lie between 330 $m\mu$ and 290 $m\mu$. The longer wave components from 400 to 330 $m\mu$ are ineffective.

The amount of energy from 290 to 280 $m\mu$, though variable, is too small to produce any definite effect. (For the mathematical analysis, see Mackenzie and Muller, 1940.) The lethal effect, on the other hand, is more nearly proportional to the total emitted on all wave-lengths, since the amount of the shorter-wave radiation is approximately constant.

The Standard Dose.—This was fixed at $2-3 \times 10^8$ ergs/cm.² with the U.G.2 filter, transmitting from 365 $m\mu$ to 280 $m\mu$. It caused the death of from 30 to 60 per cent. of the flies treated, and gave a mutation rate of nearly 5 per cent. in the remainder. It was remarkably constant for different stocks and different ages of the flies in any one stock. The irradiation of a group of flies, therefore, produces a variety of results:

- (i) Some flies perish under the influence of the radiation: this only occurs to any considerable extent if the dose is grossly excessive—some 5 to 10 times the correct dose.
- (ii) Some flies perish within 1 to 2 days of treatment. These are generally feeble and slow in their movements and unable to fly. They rarely give rise to offspring, being unable or unwilling to copulate with the females.
- (iii) Some survive for 3 to 5 days, being normally vigorous in appearance for the first day or two, and give rise to offspring.
- (iv) Others, which survive for a long period, appear to be sterile.
- (v) Some are normal in length of life and in fertility.

The proportion of these five groups was studied in more than 10,000 flies according to the dosage given. These flies were of two main types, both having yellow body colour, but in the y_2 stock the bristles were of a darker shade. The difference in response of the two groups was not appreciable, and they have, accordingly, been added together.

TABLE III.—DIRECT LETHAL EFFECT OF VARIABLE DOSES OF U.V. LIGHT ON *DROSOPHILA* MALES.

Dose of Radiation 365 $m\mu$ –280 $m\mu$ in 10^8 ergs/cm. ² .	No. of Flies Treated.	Per cent. Surviving Normal Longevity and Fertility.	Per cent. Fertile Surviving 3–5 Days.	Per cent. Sterile Dying in 3–5 Days.	Per cent. Sterile Dying in 1–2 Days.
Up to 2	600	95	3	0.2	?
From 2–3	800	89	5	1.3	1.1
3–4	2200	45.3	40.8	8.8	6.2
4–5	3600	5.1	15.3	30.8	48.6
5–6	1600	?	3.2	8.1	80.6
6–9	2000	..	0.7	?	99.0

N.B.—The above percentages do not add up to 100 per cent., owing to the disregard of flies lost or killed in irradiation, defective vials, and to the small proportion of sterile flies surviving indefinitely.

A graphical representation of Table III is shown in fig. 1. The curves of survival and mortality are approximately symmetrical, but show some relative displacement, which is accounted for by the lower two curves of fertile and sterile flies which survive for a brief period.

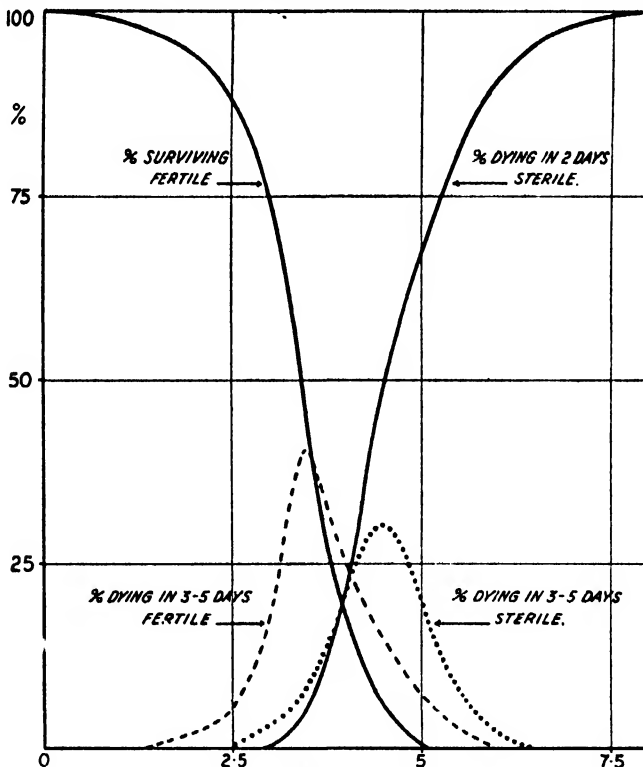


FIG. 1.—Dose in 10^8 ergs/cm.², λ 400–280 m μ .

An examination of the different groups for mutation effects shows that by far the largest number occurs in the group which only survived from 3 to 5 days.

TABLE IV.—NUMBER OF MUTATIONS FOUND IN THE DIFFERENT GROUPS SHOWN IN TABLE III.

Dose in 10^8 ergs/cm. ² (400–280 m μ).	No. of Flies Treated.	No. of Mutations in Surviving Flies.	No. of Mutations in Flies living 3–5 Days.	Per cent. of Mutations in Flies living 3–5 Days.	Per cent. of Mutations when Death Rate is included.
Up to 2	600	8	0		
2–3	800	15	8	0.3	
3–4	2200	17	35	1.8	under 1
4–5	3600	12	123	4.8	3.6
5–6	1600	..	17	6.6	3.0
6–9	2000	..	15	8.2	<0.5

In considering the per cent. mutation rate, only the values for irradiation dosages between 3 and 6×10^8 ergs/cm.² can be considered as significant. The numbers for the low doses (below 2×10^8 ergs/cm.²) would have to be increased tenfold, and with the low mutation rate obtained at this level this process would be very uneconomical. With the high doses (above 6×10^8 ergs/cm.²), the survival rate is so low (14 flies in 2000) and the raising of the F_1 so much a matter of chance, that it is not a practical procedure. Indeed, most of these results were obtained by unintentional overdosage. It emerges, therefore, that a mutation rate of more than 5 per cent. can only be obtained by great expenditure of labour and material, and, in fact, most of the genetical results obtainable in this way have already been realised, *i.e.* that translocations are not produced by this means, and that minute rearrangement of the chromosome material is very rare or absent (Mackenzie and Muller, 1940).

The following variables in the irradiation conditions have been studied:—

(i) *The Age of the Flies at the Time of Irradiation.*—A comparison of flies aged 24 hours, 3 days and 7 days or over reveals that the mutation rate is almost constant with a given dose, but that the death rate rises considerably in the older groups. The 3- and 7-day-old groups differ very little from each other. Most of the deaths in the first group seem to be due to pressure, which deforms the flies beyond the point at which they can revert to normal: possibly the intense irradiation causes their integument to harden in unnatural positions which are unfavourable to survival. But the death rate is nevertheless considerably lower than with the older flies, which suffer no direct injury by pressure. In this case it seems that the increase in pigmentation and the opacity of the chitin causes more serious injury by direct heating due to absorption.

(ii) *The Effect of Pigmentation.*—With regard to any particular variety, this question is dealt with under the head of ageing above. But when the question is applied to different varieties of the same age, the results suggest that the mutation rate is approximately constant for any given dose, while the lethal effect increases in proportion to the pigmentation. Four varieties have been studied; of these, "yellow" and "yellow₁," which differ only in the colour of the bristles, show practically similar results (it must, however, be noted that no experiment differing only in this single character has been performed, and hence the results are not strictly comparable), while "straw," a light-coloured mutant which, however, gives to the chitin or the epidermis an opaque, milky character, is almost as unfavourable as the dark-coloured "wild type" for survival. But though the percentage of survivors is so much less, the mutation

effect is almost as high, which may be an example of the "selection effect."

(iii) *Variation of the Dose*, which forms the principal topic of this paper, has already been examined.

(iv) *The Subsequent Survival Rate of the Flies* appears to be a simple function of the dose. At low levels the majority survive; at the optimum dose, survival is moderate, and the mutation rate is low in the survivors; at high doses, practically the whole population dies.

DISCUSSION.

It has been shown that the effect of ultra-violet light on *Drosophila* begins to be appreciable at a fairly large dose, the effects being absent, or at least not detectable, at less than 10^7 ergs/cm.² of the effective wavelengths, which lie below 330 mμ. From this point the gross mutation rate increases with the dose for a factor of two or three and then as rapidly declines. This gross mutation rate, the percentage of mutation found in the whole group of treated flies, must be distinguished from the *net* mutation rate found in the flies which survive the treatment for a period long enough to show fertility—a period of three to five days. The net mutation rate shows a steady increase with dose as far as it can be followed, *i.e.* to the point where there are sufficient flies surviving to give a significant result.

The direct lethal effect of the ultra-violet irradiation begins at a somewhat higher level than the mutation effect, and increases more rapidly than the latter. When the mutation rate has reached 5 per cent., the lethal rate is 50 per cent., and before the net mutation rate can reach 10 per cent., the lethal rate reaches 100 per cent. Fig. 2 shows the curves of net mutation, gross mutation, and mortality in relation to each other.

It remains uncertain whether the mutation effect and the lethal effect are independent of each other, but the similarity of the curves and their close approach to each other makes it seem likely that there is some connection between them.

If the assumption is made that a single quantum of ultra-violet light can bring about a mutative change in the chromosome, it is possible to make a rough calculation of the probability of any single quantum producing this result as follows:—

2.5×10^7 ergs/cm.² produce 5 mutations per 100 flies.

Assuming a wave-length averaging 320 mμ, the conversion factor is

$$h_{320} = 6.15 \times 10^{-12} \text{ ergs,}$$

so that, roughly,

$8 \times 10^{10} \text{ h/cm.}^2$ produce 1 mutation per fly.

If we assume the sperm in which the mutation is brought about is 1μ long and 0.1μ wide, its area is 10^{-9} cm.^2 , and the number of quanta falling on this area for the effective dose is

$$8 \times 10^{10}.$$

The probability of any one quantum producing a mutative change is therefore less than one in ten thousand million. This seems a large

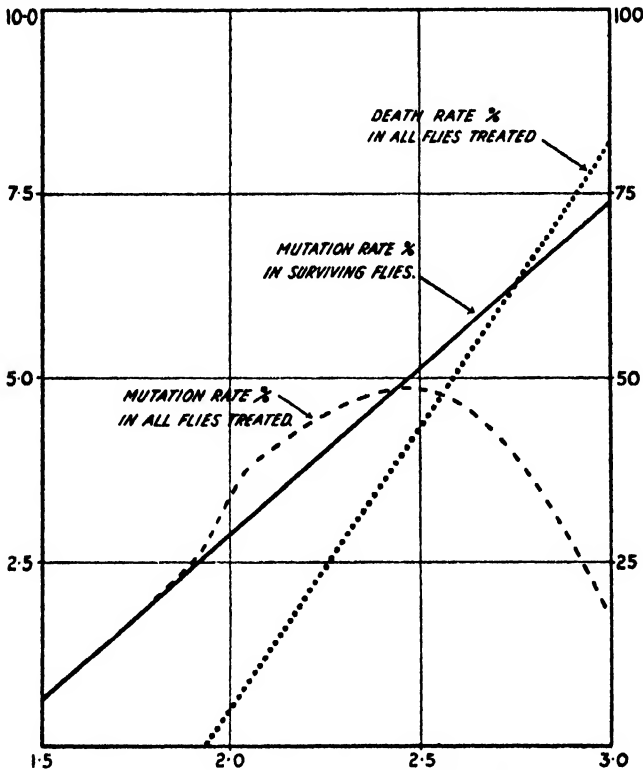


FIG. 2.—Dose in 10^7 ergs/cm.^2 , $\lambda \text{ 330-280 m}\mu$.

number, but it must be remembered that the spermatogenic material is surrounded by a filtering medium, chitin, of almost perfect opacity. Were it not so, the effect of sunlight, to which the flies are frequently exposed, would be in itself a fruitful cause of mutations, which actually occur with a very low frequency. The filtering properties of the chitinous envelope of *Drosophila* are therefore of great advantage in protecting them from the effects of natural ultra-violet irradiation.

SUMMARY.

1. Ultra-violet light, in optimum dosage, produces about 5 per cent. of mutations in *Drosophila*.
2. The probability of mutation per quantum is of the order of 3×10^{-10} at 320 $m\mu$.
3. Higher doses than the optimum are accompanied by considerable mortality.
4. The mutation effects and the lethal effects appear to be linked. The mutation effect is proportional to the dosage of the wave-lengths 330-290 $m\mu$.

My thanks are due to the Scottish Cancer Control Organization for a grant in aid of this work; to Professor F. A. E. Crew for accommodation in the Institute of Animal Genetics; and to Dr H. J. Muller and Dr A. C. Aitken for advice on certain points.

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* By an error, the energy measurements in this paper are given as 10^3 times the correct figure.

(Issued separately July 25, 1941.)

VII.—The Genetical and Mechanical Properties of the Sex Chromosomes. VIII. The Cat (*Felis domestica*). By P. C. Koller, Ph.D., D.Sc., Institute of Animal Genetics, University of Edinburgh. (With Twenty-seven Text-figures.)

(MS. received February 11, 1941. Read June 2, 1941.)

INTRODUCTION.

A CYTOLOGICAL study of the testes of the cat was undertaken in order to disclose the structural differentiation of the sex chromosomes. It was hoped that an analysis of these particular chromosomes in the male sex during meiotic division might reveal structural peculiarities which would throw some light upon the highly complex genetical behaviour of tortoise-shell cats.

MATERIAL AND TECHNIQUE.

Testicular material from a number of tabby, black, and yellow cats was fixed in Minouchi-A solution. Sections were cut at 16–20 μ thickness and stained with gentian violet. Through the kindness of the Royal (Dick) Veterinary College, Edinburgh, testes of sterile tortoise-shell males were also obtained, but they were undifferentiated, and spermatogonial cells and primary spermatocytes were entirely absent.

Drawings were made with the aid of a Zeiss Camera Lucida, and the magnification of text-figures is about 2700.

CHROMOSOME NUMBER AND STRUCTURE AT MITOSIS.

The diploid chromosome number in the metaphase of the spermatogonial division was counted in several cells and found to be 38 in the male sex of the various mutant types. This number is the same as that found previously by Minouchi (1928), Minouchi and Ohta (1934), and Matthey (1934).

The chromosomes at mitotic metaphase exhibit a variation in size. The largest chromosome pair is about 6–7 μ long, and the smallest 2–2.5 μ . The shape of chromosomes during metaphase indicates the position of the centromere. It was found that most of the chromosomes are V-shaped with equal or unequal arms. The centromeres of the larger chromosomes have a subterminal or submedian position, while the locus of the centro-

mere in the smaller chromosomes is median (figs. 1-3). The configuration of bivalents during meiotic metaphase indicates that there are two arms in each of the chromosomes. According to Matthey (1936) the centromere

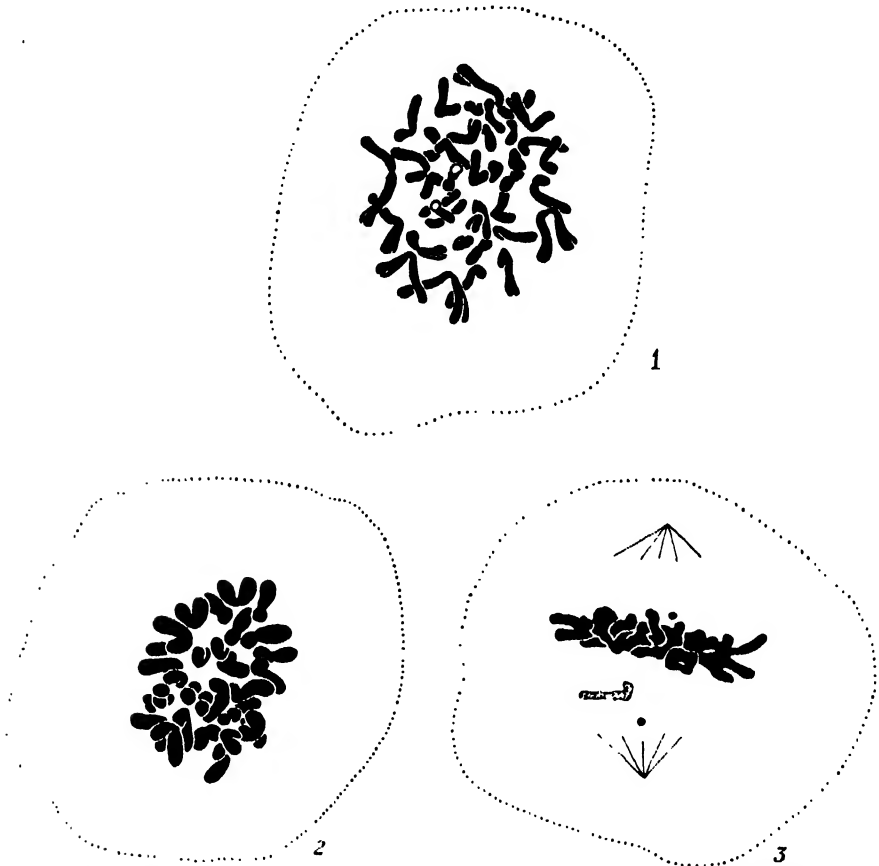


FIG. 1.—Metaphase of mitosis in the spermatogonia. The chromosomes differ in size and shape. The sex chromosomes cannot be distinguished.

FIG. 2.—Mitotic metaphase, showing a strong contraction of chromosomes.

FIG. 3.—Mitotic metaphase, showing the two plasmosomes and a chromosome which is stained lightly and lying off the equatorial plate.

in the 5 smallest pairs is terminal, but in the types investigated here these small chromosomes have two arms.

During the study several spermatogonial cells were encountered in which the chromosomes in the metaphase were much more contracted than in adjacent spermatogonia (fig. 2). They always appear in groups, indicating that the peculiarity is derived from one cell in which the genotypic control is altered by mutation. The mechanical and genetical

implications of such a change in chromosome behaviour is discussed by Darlington (1932).

Side view of metaphase in several spermatogonial cells has shown that frequently a varying number of chromosomes are lying off the equatorial plate. In this respect the large and small chromosomes behave in a similar way. Such a delay in the congression of chromosomes on the equator is attributed to the incompletely developed spindle mechanism which affects the free movement of chromosomes (Darlington, 1939).

In one spermatogonial cell, showing metaphase, a chromosome was seen lying off the equator, which was slightly stained with gentian violet compared with the others within the same cell (fig. 3). It is not improbable that this particular chromosome is in a state of degeneration due to some defect in its internal structure. In the cytoplasm of adjacent cells a heteropycnotic body was seen which also suggests the elimination of a chromosome from the diploid complement of some spermatogonial cells.

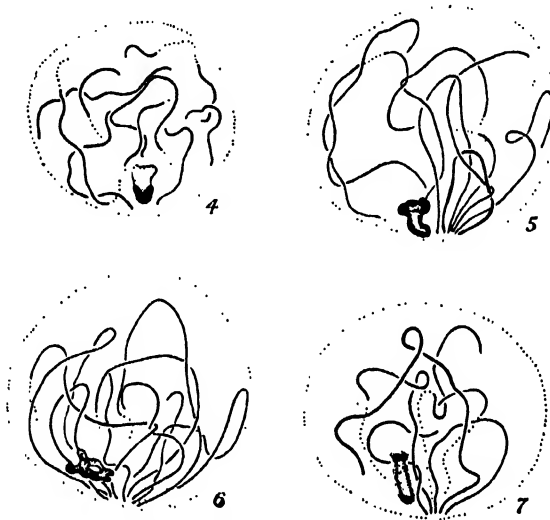
During the present study two small round-shaped "plasmosomes" were identified as a permanent nuclear constituent which appears during mitotic prophase, metaphase, and anaphase (fig. 3). They exhibit a very variable staining ability; in some cells the plasmosomes remain almost unstained, while in others they are as deeply stained as the chromosomes.

The chromosomes of the diploid complement can be arranged in a series of 19 pairs. A comparison of chromosomes has shown that a "heteromorphic" or unequal chromosome pair, which would represent the X and Y sex-chromosomes, is absent, which suggests that the two sex-chromosomes are apparently very similar in size. It must be pointed out, however, that in the diploid chromosome complement of the cat there is a gradual decline between the largest and smallest chromosome pair in respect of length, which makes it very difficult to identify a particular chromosome pair the members of which are slightly different in length. Probably this fact accounts for the mistake made by Winiwarter and Sainmont (1909) in stating that the male sex in the cat has only one sex chromosome. A further analysis of chromosome behaviour during the meiotic division is therefore necessary in order to identify and determine the structure of the sex chromosome in the cat.

THE BEHAVIOUR OF SEX CHROMOSOMES DURING MEIOSIS.

Amongst the fine threads which represent the chromosomes in the early prophase nucleus of primary spermatocytes, one to three, and

occasionally four, deeply stained structures of varying shape and size can be recognised. In the pachytene stage only one such body is present which is called "nucleolar body." The pachytene stage is interrupted by a long period of growth when the volume of the nucleus increases.



FIGS. 4-7.—Meiotic prophase. The autosomal chromosome threads are polarised and the precociously condensed XY complex is seen lying near the polarisation centre. The sex chromosome complex shows a definite bipartite structure during the pre-growth prophase stages (fig. 5).



FIG. 8, *a*, *b*.—The various types of XY (*a*) before, and (*b*) after, the growth period of meiotic prophase.

During this period the threads, representing the paired homologous chromosomes, stain very lightly with gentian violet, while at least a portion of the characteristic "nucleolar" body is deeply stained, indicating regional differences in its internal organisation (figs. 4, 5, 6, 7). It has been definitely established, by following the behaviour of this particular body during the successive stages of meiosis, that it represents the associated XY sex-chromosome complex. Matthey (1936) has also

reported the presence of a bi-partite "karyosome." It was found that this nucleolar body, or the XY complex, exhibits great variation in shape during the earlier stages of meiosis. As judged from the staining, the

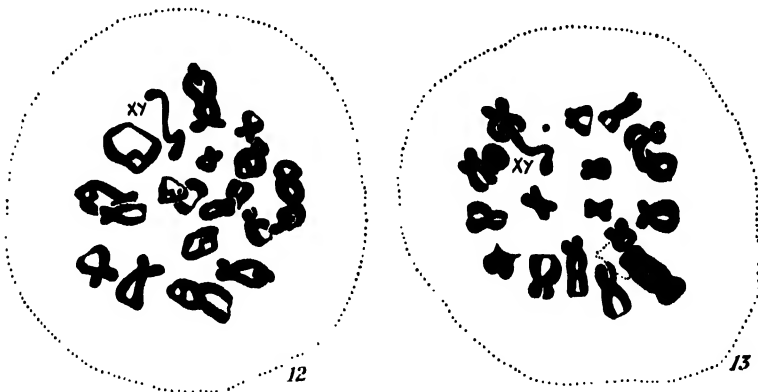


FIGS. 9-10.—Nuclei of primary spermatocytes, showing late diplotene stage. The XY bivalent can easily be identified by its shape and by the regional differences in condensation.

FIG. 11.—Diakinesis, showing the thread-like XY bivalent.

regional differences in the degree of condensation are also very variable, but usually one terminal region is condensed while the remainder is in a more or less diffuse stage (fig. 8).

The autosomal chromosome threads are polarised at the pachytene



FIGS. 12-13.—Polar view of first meiotic metaphase, showing the position and configuration of the sex chromosomes which are associated by terminal chiasma.

stage (Winiwarter, 1914), and the XY complex lies near to the polarisation centre (figs. 6 and 7). During the various stages of meiosis no association between the sex-chromosome complex and autosomal chromosomes was found.

At the late diplotene (figs. 9 and 10) and diakinesis (fig. 11) stages of meiotic prophase the autosomal bivalents are evenly distributed within the nucleus. While the XY complex forms a thick bulky body during

the earlier stages, here it is represented by a long and somewhat thin lightly stained thread easily distinguishable from the characteristic looped configuration of the autosomal bivalents. The difference between autosomal and sex bivalents is more accentuated at metaphase (figs. 12, 13). Apart from a delay in condensation of the sex chromosomes as compared with the autosomes, there are regional differences within the XY bivalent itself: usually the terminal regions are thicker (figs. 9, 10), indicating that they are more contracted than the intercalary segments; the variability in shape, size, and staining capacity is very probably attributable to a lack of co-ordination in respect of condensation and contraction of the different regions. The underlying basis may be found in the different distribution of the nucleic acid content within this particular chromosome (Darlington and La Cour, 1940).

The analysis of the structure of autosomal bivalents has shown that the average number of chiasmata per bivalent is 2.7 and 2.4 at diakinesis and metaphase respectively (Table I). The data indicate a slight insignificant decrease in the number of chiasmata from diakinesis to metaphase.

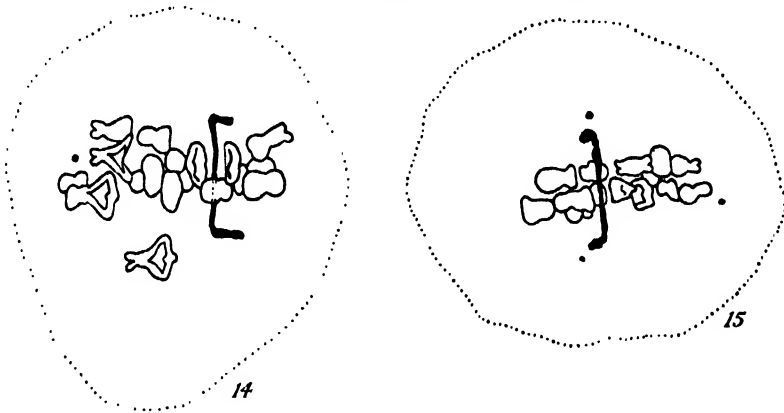
TABLE I.—CHIASMA FREQUENCIES AT DIAKINESIS AND METAPHASE.

Chiasmata	1.	2.	3.	4.	Total No. of Chiasmata.	Total No. of Terminal Chiasmata.	
Diakinesis	..	10	5	3	47	12	
	..	8	7	3	49	15	
	..	10	6	2	46	16	
	..	7	8	3	50	14	
	..	9	7	2	47	15	
	..	6	10	2	50	12	
	..	5	10	3	52	14	
	..	55	53	18	341	98	Total No. of Bivalents. 126
the variance = 0.4932							Chiasmata per Bivalents 2.7
Metaphase	..	13	3	2	43	18	
	..	12	4	2	44	19	
	..	14	3	1	41	15	
	..	10	6	2	46	21	
	..	11	6	1	44	22	
	1	12	4	1	41	18	
	1	72	26	9	259	113	Total No. of Bivalents 108
the variance = 0.4289							Chiasmata per Bivalents 2.4

$$\begin{aligned} \text{Test of significance of difference} &= +0.3 + \sqrt{0.4932 + 0.4289} \\ &= +0.3 \pm 0.960. \end{aligned}$$

The presence of diffuse segments and the failure of the different parts of the sex-chromosome complex to co-ordinate their condensation and contraction make it difficult to ascertain the method of association of the X and Y at the initial stages of meiosis. It was assumed on analogy (Koller, 1937) that they are associated by chiasmata, and that the number of chiasmata is usually one. Few primary spermatocytes were found in which the X and Y chromosomes are associated by two terminal chiasmata.

In various animals it was seen that autosomal bivalents, having only one terminal chiasma, are held together by a long thin thread which



FIGS. 14-15.—Side view of first meiotic metaphase. The XY bivalent is drawn solid black. Two types of XY can be recognised.

suggests that there is a strong centromere repulsion between the homologous members of the bivalent (Darlington, 1937). The two associated sex chromosomes appear as a long thread of even thickness along the whole length, so that the place of the terminal chiasma could not be recognised (figs. 12, 13, 14). In some XY the regions adjacent to the terminal chiasma are thinner (figs. 14 and 15), but never to such an extent as is the case in autosomal bivalents.

An attempt was made to determine (1) the position of the centromere, (2) the length of the two sex chromosomes, and (3) their internal organisation. The side view of the meiotic metaphase has shown variation in respect of the length and shape of the XY bivalent (figs. 14, 15). The members of the sex bivalent have two limbs; one is associated by a terminal chiasma, the other is free from chiasmata. The position of the centromere is indicated by a more or less easily recognisable bend in the outstretched sex bivalent. In several primary spermatocytes it was seen that the free limbs of the XY differ in length, while the regions

which lie between the centromere and terminal chiasma are equal. Furthermore, it was found that the chiasma-free arms are shorter than the other arms (fig. 14). Another type of XY bivalent was also encountered in which the arms between the centromere and the chiasma differ slightly, while those with no chiasma are of the same size and longer than the former (fig. 15). These two types are asymmetrical

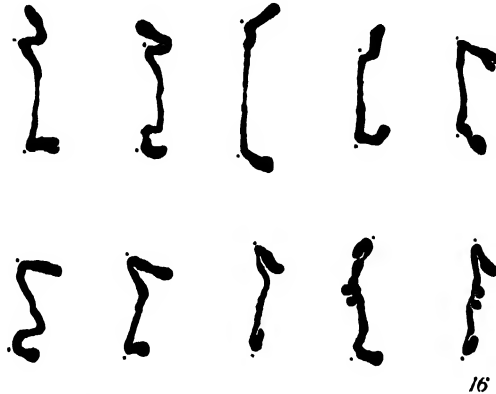


FIG. 16.—XY bivalents of 10 primary spermatocytes, showing chiasma in the long arm of X and Y. The position of the centromere is indicated by a dot. The last two XY bivalents have a subterminal chiasma, while the others are associated by a terminal chiasma. The free arms are of unequal size.



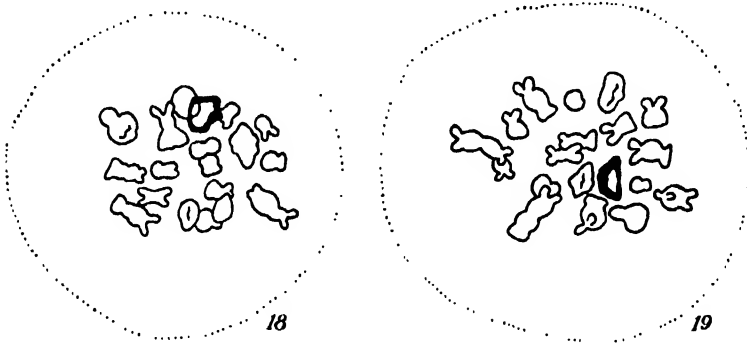
FIG. 17.—XY bivalents of the first meiotic metaphase, showing chiasma formed in the short arm of X and Y: the chiasma-free arms are of equal size.

(figs. 16, 17) and suggest that the X and Y chromosomes have a submedian centromere which divides the chromosomes into a long and a short arm. The shape of XY indicates that the length of the long arm is similar in X and Y, but the short arm of the Y is slightly smaller than that of the X. During the present study only 2 (possibly 3) XY bivalents, out of 134, showed a knob in the associated arm, which may be interpreted as a subterminal chiasma (fig. 16).

In view of the fact that there are two terminal homologous or pairing segments in the X and Y, it was expected that instead of the rod-shaped XY, a ring-shaped sex bivalent might be formed occasionally as a result of simultaneous chiasma formation in both pairing segments. This ring-shaped bivalent represents the third type of XY, and was actually

recognised in 4 out of the total 134 primary spermatocytes analysed (figs. 18 and 19).

The three types of XY bivalent observed at metaphase enables us to



FIGS. 18-19.—Metaphase of meiosis, showing the third type of XY bivalent which is the result of chiasma formation in both arms.

determine the internal differentiation of the two sex chromosomes. They are composed of a long and a short arm; each arm has a pairing segment (fig. 20). The association of X and Y being most frequently by a terminal chiasma, it is assumed that the pairing segments are terminal. The

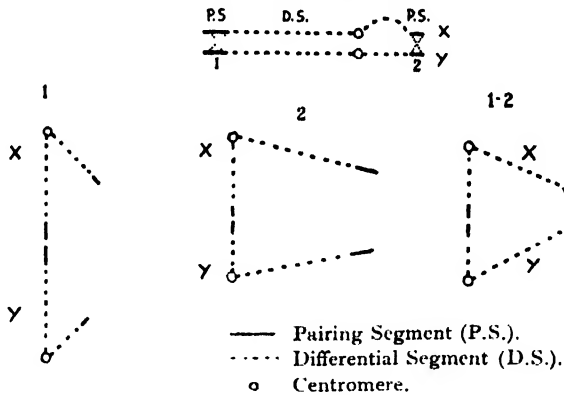


FIG. 20.—Diagram illustrating the internal differentiation of the sex chromosomes in the cat. The configuration of metaphase XY bivalent is determined by the position of chiasma (at 1, 2, or 1 and 2).

very low frequency of subterminal chiasmata indicates that the terminal pairing segments are very small and every chiasma which is formed, being next to the end, can terminalise before metaphase in spite of the weak centromere repulsion characteristic of the XY bivalent. In about two-thirds of the XY bivalents analysed, chiasma formation has taken place in the long arm, from which it is assumed that the homologous

segment of the long arm of X and Y is larger than that of the short arm.

The segment which carries the sex-differentiating gene system (hence called the differential segment) is located interstitially in the X and Y chromosome of the cat. This segment is excluded from pairing and crossing-over. Analysis of the various types of XY suggests that the centromere lies within the differential segment. It was postulated by Koller and Darlington (1934) that when the centromere is located within that segment, a pre-reductional segregation of structural differences of X and Y is obligatory during meiosis. The configuration of XY at metaphase shows that the differential segment of the short arm differs in the X and Y; it is somewhat larger in the X chromosome.

The structural differentiation in the sex chromosomes of the cat is further evidence which proves that the metaphase configuration of the XY bivalent is not always an indication of the method of reduction (Koller, 1941). The ring XY bivalents, owing to the small differences in size between X and Y, are symmetrical, but the first meiotic division remains reductional in respect of the structural differences, as it is the case in the asymmetrical XY bivalents.

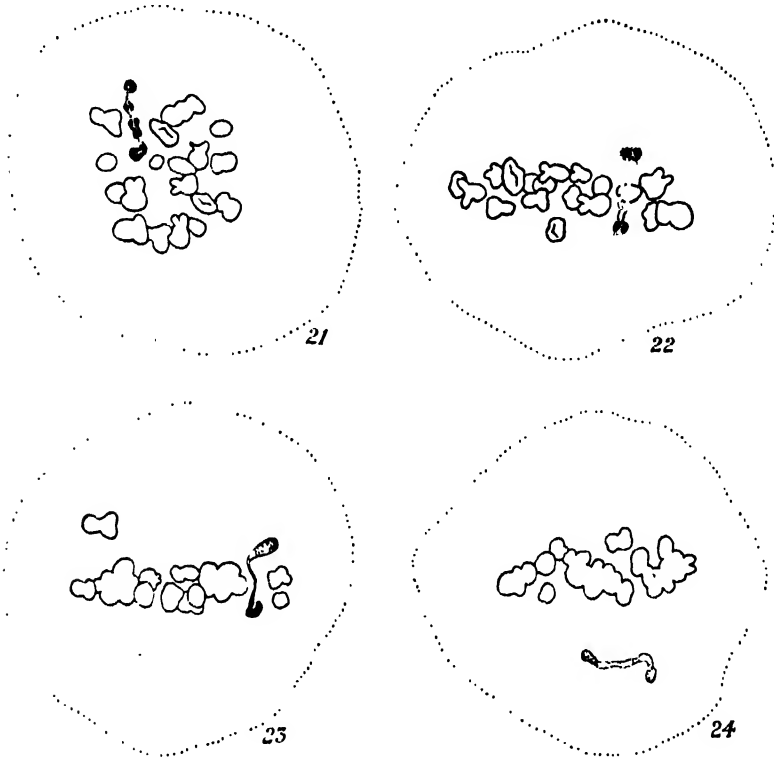
It is of interest to note that no other mammal is known in which the differential segment of the sex chromosomes is located interstitially; only in the sex chromosomes of *Phragmatobia* (Lepidoptera) is a similar structure reported by Seiler (1925), and it is now known that in the complex heterozygote *Oenothera*, the differential segments are also interstitial (Darlington, 1931).

IRREGULAR BEHAVIOUR OF THE SEX CHROMOSOMES.

It is believed that the degree of staining of a chromosome can be taken more or less as an expression of the degree of *condensation* within the chromosome. During the mitotic and meiotic divisions the autosomes exhibit a uniform condensation; not only does every autosomal chromosome show the same degree, but every region within the chromosome itself behaves in the same way. In the sex chromosomes, however, there are regional differences in this respect (figs. 21-24, and 26). It has already been mentioned that during the earlier stages of meiosis the XY complex is composed of condensed and diffused regions, the size and position of which vary greatly: the distal, and occasionally the proximal, regions of the XY bivalent in the cat show a lack of condensation at diplotene, diakinesis, and metaphase, as compared with the other regions. The failure of segments to co-ordinate their functions during development

has been observed in several organisms and it is regarded as a characteristic property of the sex-chromosomes. One may conclude that the sectional differences in condensation are the morphological expressions of an internal differentiation of these particular chromosomes, and represent the genetically active and inert regions (Darlington and LaCour, 1940).

It was found that contraction is irregular in the XY bivalent, some-

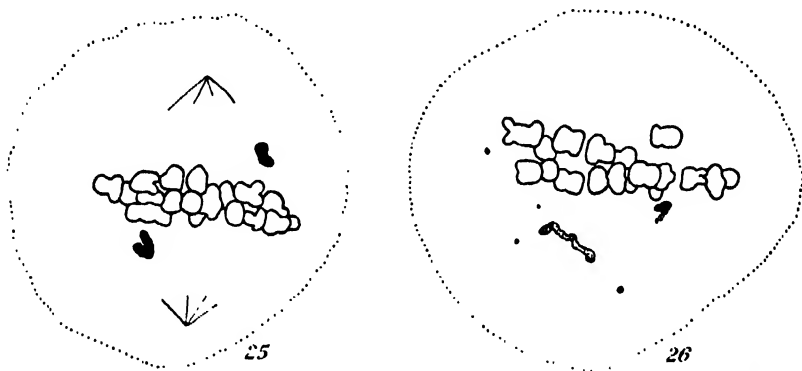


FIGS. 21-24.—First meiotic metaphase, showing irregular behaviour of the sex bivalent.

times one chromosome or part of a chromosome being more contracted than the other. While the XY complex contracts precociously during pachytene, its contraction is delayed during the post-diplotene stages, in comparison with that observed in the autosomes. It is accepted now that contraction of a chromosome is brought about by spiralisation (Darlington, 1937). Contraction may be due (1) to an increase in the number of spirals, (2) to an increase in the diameter of the spirals, or (3) to a decrease in the distance between two adjacent spirals. It is very probable that all the three processes participate. Consequently one may

assume that the irregularities of sex chromosomes in respect of contraction are due to a lack of co-ordination of these processes. The lack of contraction is usually accompanied by the lack of condensation in the particular chromosome. Several instances were, however, found in which the non-contracted XY bivalent is condensed, while in others the contracted XY bivalent shows regions in a diffuse stage.

During the division cycles the centromere of the sex chromosome was found to exhibit a behaviour which is very different from that observed in the autosomes. While the arms of autosomal rod-shaped bivalents, which have a terminal chiasma, are always directed towards



FIGS. 25-26.—First meiotic metaphase, showing unpaired sex chromosomes.

the opposite poles, the associated arms of X and Y usually bend and show coiling. Such a configuration at the metaphase clearly indicates that the repulsion between the centromeres of the two sex-chromosomes is weak or delayed. A study of first meiotic anaphase has also shown that centromere-repulsion, which is primarily responsible for the segregation of the members of a bivalent, is delayed and the sex bivalent is left lagging on the equator. Furthermore, it was found that when XY bivalents lie off the equatorial plate during metaphase, they fail to orientate themselves towards the poles (fig. 24), and when unpaired they are either pushed off the equator or fail to move into the equatorial plate (fig. 25). These irregularities show that the function of the centromere in the X and Y chromosomes is interfered with and that there is a definite lack of co-ordination between the function of centromere and centrosome.

DISCUSSION.

Analysis of sex chromosomes in various mammals has shown that the internal differentiation of the X and Y exhibits a great variation which

represents the different stages of evolution in the sex-determining mechanism (fig. 27).

In the course of earlier investigations concerning the sex chromosomes in various organisms it was definitely established that these chromosomes are composed of a pairing and a differential segment—the latter being the region in which the completely sex-linked genes and the sex-differentiators themselves are located (Koller and Darlington, 1934).

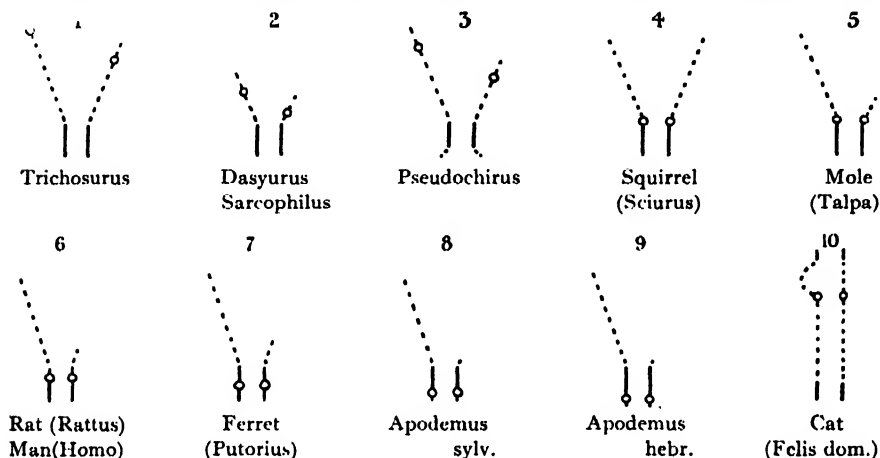


FIG. 27.—Diagram showing the various kinds of differentiation in the sex chromosomes in different organisms. The X chromosome is on the left. Parts indicated as in diagram 20.

The relative length of the two segments and their position in relation to the centromere varies in different organisms.

The position of the centromere is of special importance because it determines the method of segregation of the XY bivalent during first meiotic anaphase. When the centromere is located in the differential segment, pre-reduction is obligatory; when, however, it is in the pairing segment, pre-reduction of structural differences is facultative and will depend upon the loci of chiasma or chiasmata formed in the homologous segment. If we accept the view that there is a direct proportionality between chiasma frequency and chromosome length (Darlington and Dark, 1932), the frequency of the various types of XY bivalent at metaphase indicates the relative length of the pairing segments in the cat. Our data suggest that the pairing segment of the long arm is longer than that of the short arm.

The great variation in the size of the differential segment of the Y chromosome, which was observed in several organisms, is due to the fact that it is excluded from pairing and crossing-over in the sex

heterozygote. In *Apodemus* (fig. 27, Types 8 and 9; Koller, 1941) this segment is represented by a few genes only, while in *Trichosurus* (fig. 27, Type 1; Koller, 1936) it is at least three times as large as the pairing segment. The various types of the sex chromosome suggest that if the centromere is localised within the pairing segment, the differential segment of the Y will decrease and may ultimately disappear in the course of evolution. The Y chromosome of the squirrel (fig. 27, Type 4) is apparently an exception: the differential segment of X and Y is similar in length in spite of the peculiar position of the centromere, which is located at the point where the two segments join. If the centromere is in or near the free end of the differential segment, inversion may change its position, placing the centromere next to the pairing segment. The gradual reduction of the differential segment in the Y chromosome is conditioned by each change in the position of the centromere. It is not improbable that a change in the position of the centromere in the sex chromosomes of the squirrel has taken place recently.

The shape of the rod-XY bivalent in the cat indicates that the differential segment of the short arm of X is longer than that of the Y chromosome. This cannot be due to the different position of the centromere in the X and Y alone. In view of the fact that the long arm of the two sex chromosomes is equal in size, the difference in the length of the short arm cannot be attributed to an inversion including the centromere, but it is more probably the result of a deficiency. Such structural change could easily have occurred and been perpetuated owing to the heterozygosity of the differential segment. If this is the case we may consider the male sex in the cat as a deficiency heterozygote. On the other hand, it is not impossible that the slight difference in size may be due to a lack of co-ordination of the different parts of X and Y in respect of their various functions, particularly that of contraction.

It has been argued that structural changes are not the primary agents in the suppression of crossing-over and in the conditioning of sex-differentiation (Darlington, 1939). Gene mutation or mutations are the initial step in the evolution of the sex-determining mechanism, which is followed by crossing-over suppression in the region where sex-differentiating genes are located. It is known now that suppression of crossing-over not only isolates chromosome regions, but also leads to inertness of these particular segments (Muller, 1918). The differential segment of Y, because of its heterozygosity, is the part of the chromosome which is most affected in this respect. Further structural changes leading to the reduction and ultimately to the complete elimination of this region, can easily occur without endangering the genic balance and viability of the

sex heterozygote. The great variation in size of the differential segment of the Y chromosome observed in previous studies is a definite proof that such changes have actually occurred in the sex-determining mechanism of many organisms. If, however, the centromere is included in the differential segment as is the case in the cat, the reduction in size and the elimination of this region is greatly hindered or delayed, and as a result several qualitative and quantitative changes may be able to accumulate in this particular region.

The structural peculiarities of the sex chromosomes, particularly those of the Y, probably form the physical basis of the irregular genetical behaviour observed in the tortoise-shell cat. Tortoise-shell is a heterozygous type and it is produced by mating yellow with black. It was reported by Doncaster (1904) that tortoise-shell males are extremely rare and when they do occur they are nearly always sterile. Doncaster (1912) and Tjebbes and Wriedt (1926) suggested that crossing-over between the X and Y chromosomes may account for the occasional fertility of tortoise-shell males. This suggestion, however, was contested by Bamber and Herdman (1937) on purely genetical grounds.

It is not improbable that the low frequency of tortoise-shell males, their rare fertility, and the occurrence of exceptional black females from yellow males (Bamber, 1927) are all related phenomena. At present, however, it is not feasible to formulate a hypothesis based upon the structural peculiarities of the sex chromosomes alone which would satisfactorily explain the physical basis underlying the genetical behaviour of these exceptional types. We must assume that the genic contents of the sex chromosomes constitute highly complex complementary systems and that the various units of complementary gene systems, concerning viability and fertility (or sterility), are distributed in the pairing and differential segment. Crossing-over in the pairing segments of X and Y necessarily leads to the disruption of these complementary systems. It is not improbable that there exist various X and Y chromosomes which differ in respect of the genes constituting these complementary systems. A particular kind of combination of the sex chromosomes would account for the exceptional types.

SUMMARY.

1. The diploid chromosome number is 38 in the cat (*Felis domestica*). The centromeres of the larger chromosomes have a subterminal or submedian position; it is median in the smaller chromosomes.
2. A comparison of chromosomes of the diploid complement shows that

an unequal chromosome pair is absent, which suggests that the X and Y sex chromosomes are very similar in size.

3. Three types of XY bivalents are identified at meiotic metaphase. The first two types are rod-like and asymmetrical, having one terminal chiasma; the third type is a ring-shaped sex bivalent and has two terminal chiasmata.

4. The metaphase configuration of the XY bivalent indicates that the sex chromosomes have a submedian centromere which divides the chromosomes into a long arm and a short arm. Both arms have a terminal pairing segment. The differential segment is intercalary and includes the centromere. In the short arm of Y it is smaller than that of the X chromosome and accounts for the slight difference between these chromosomes in length.

5. The sex bivalent exhibits several irregularities such as lack of co-ordination in condensation and contraction. Repulsion between the centromere of X and Y is delayed at meiotic metaphase, and a lack of co-ordination between the function of centromere and centrosome was also observed.

6. It is suggested that the structural peculiarities of the sex chromosomes form the physical basis of the complex genetical behaviour observed in the tortoise-shell cat.

I acknowledge with thanks the award of a grant from the Carnegie Trust for the Universities of Scotland towards the cost of the tables and illustrations in my paper.

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94 *Genetical and Mechanical Properties of the Sex Chromosomes*

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VIII.—The Structural Differentiation of Chromosome IV of *Drosophila simulans* and its Behaviour in *melanogaster* Genotype. By B. M. Slizynski, Ph.D., Institute of Animal Genetics, University of Edinburgh. Communicated by Dr P. C. KOLLER. (With Five Text-figures.)

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I. INTRODUCTION.

THE problem of viability and fertility of interspecific hybrids in *Drosophila* has been attacked most recently by Muller and Pontecorvo (1941). They crossed diploid *Drosophila simulans* males with triploid *melanogaster* females, and, by using a specially designed technique, succeeded in introducing into the marked genotype of *melanogaster* some chromosomes of *simulans*. Amongst the many interesting results obtained, it was found that not all interspecific combinations of chromosomes were viable; the large chromosomes of one species show a definite "need" for some genes or group of genes in other chromosomes of the same species, and these authors have drawn the conclusion that there are a number of complementary "lethals" in both species located in the major chromosomes (X, II, and III).

The ratio of lethal chromosome combinations to sterile ones shows that the number of complementary factors causing sterility is higher than that of those responsible for lethality. The partial hybrids obtained by Muller and Pontecorvo were all sterile with the exception of one female, which had all its major chromosomes from *melanogaster*. When this female was bred, tests of the offspring showed that the chromosomes Y and IV of *simulans* had been transferred into the *melanogaster* genotype, though apparently there were no visible phenotypical effects of such a chromosome transference. During the present work, it has been found that the number and size of the hairs arranged in transverse rows on the pupal case are influenced somewhat by the presence of a chromosome IV from *simulans*, but the data are too meagre to allow us to draw any definite conclusion.

This is the first successful attempt to transfer chromosomes from one *Drosophila* species to the genotype of another without impairing the fertility of the resulting partial hybrid.

Drosophila melanogaster × *simulans* partial hybrids derived from this female were used in the present cytological analysis, which is chiefly concerned with comparative study of the structural differentiation of the *simulans* chromosome IV and with its behaviour in the *melanogaster* genotype.

II. MATERIAL.

The material consists of two stocks, A and B. Stock A, in respect of chromosome constitution, has a *melanogaster* genotype, with the exception of the small chromosome IV, one of which is from *simulans*, and the other from *melanogaster*. Stock B is triplo-IV, having two MIV and one SIV.

The following symbols are used in the text: X, Y = sex chromosomes; R, L = right or left arm of a chromosome; II, III, IV = the three autosomal chromosome pairs; M, S = *melanogaster* or *simulans* chromosomes; sp = speck, a recessive autosomal character causing darkening of axillæ of the wings, located in MIIR at 107·0; e = ebony, a recessive autosomal character causing black body-colour, locus MIIR, 70·7; Cy = curly wing character connected with large inversion in MIIR and MIIL; apl = several recessive genes in MII; ci = *cubitus interruptus*, a recessive character causing several gaps in vein of the wing, located in MIVR at 0·0; ey = eyeless, recessive character reducing the size of the eye, located in MIVR at 0·2.

Both stocks originated from a cross between the original yellow, brown, ebony female hybrid and a *melanogaster* male of constitution $\frac{Cy\ sp}{apl} / \frac{ci\ ey}{ci\ ey}$. The offspring obtained from the mating are enumerated in Table I.

TABLE I.

		Females.	Males.	Totals.	
Eyeless	Curly	2	3	5	10
	Non-curly	3	2	5	
Non-eyeless	Curly	3	5	8	15
	Non-curly	1	6	7	
Total		9	16	25	

The ratio of eyeless (10) to non-eyeless (15) flies is in agreement with expectation based on the parental formulæ given. Amongst the offspring there were triplo-IV individuals which formed the foundation of stock B. Their presence suggests that the original parental female was also

triploid; she may have had one SIV and two MIV, one or both of the MIV carrying the gene for eyeless. Since, however, non-disjunction of the chromosomes IV in a diplo-IV hybrid is necessarily facilitated by the presence of a relatively large inversion in the middle region of the SIV, it is more probable that the parental female was diplo-IV, of the composition MIV/SIV.

III. METHODS.

The salivary glands were dissected from mature larvæ in acetocarmine stain—using stainless needles and forceps—by holding the larva at the caudal end with forceps and subsequently disrupting it with a needle put just in front of the jaws. This method secures quick and efficient isolation of the salivary gland and frees it almost entirely from the fat bodies.

The alcohol-euparal technique, as described by Bauer (D.I.S., No. 6), was employed in making permanent slides.

All drawings were made using an aplanat condenser 1.4 N.A., a 90× oil immersion objective of 1.3 N.A., and for drawing of general outlines a 30× eyepiece with camera lucida at table level (tube length 170 mm.), giving the magnification of 5000 diameters. Exact details were drawn in, using the same optical outfit with 10× or 15× eyepieces.

The Bridges' reference system has been applied, as published in 1935.

IV. MORPHOLOGY AND BEHAVIOUR OF THE CHROMOSOME IV IN *MELANOGASTER* AND *SIMULANS*.

38 larvæ were dissected. The type of chromosome constitution and the sex ratio are shown in Table II. The larger number of females is probably due to the fact that the male larvæ grow more slowly than the female, and consequently a greater number of females were found and selected in the culture for cytological observation.

TABLE II.

Sex Type.	MIV/MIV.	MIV/SIV.	MIV/MIV/SIV.	Totals.
Females	9	10	5	24
Males	8	4	2	14
Total	17	14	7	38

Larvæ of the constitution MIV/MIV/SIV are represented by 7 individuals; these are derived from stock B. Larvæ which were partial hybrids and diplo-IV's were derived from both stock A and stock B.

In respect of the chromosome IV, three kinds of larvæ were encountered during the cytological analysis: (1) larvæ with two *melanogaster*

chromosomes; (2) larvæ with two *melanogaster* and one *simulans* chromosomes (fig. 1); (3) larvæ with one chromosome IV from *melanogaster* and one from *simulans* (fig. 2). In larvæ of type MIV/MIV the chromosomes IV were found to pair and show a strong tendency to stick in the chromocentre mass at both ends, in the form of a C.

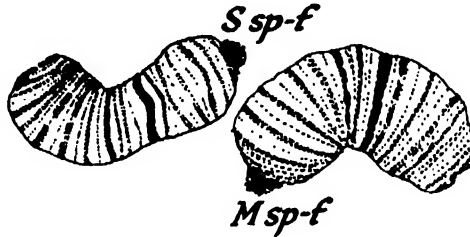


FIG. 1.—Right arms of two paired chromosomes IV of *Drosophila melanogaster* and of a single chromosome IV of *Drosophila simulans* in a salivary gland nucleus of a partial hybrid larva. S sp-f denotes proximal end of a *simulans* chromosome and M sp-f that of *melanogaster*. Drawn at $\times 5000$, reduced at $2/3$.

In larvæ of MIV/MIV/SIV constitution, the two MIV's exhibit the regular behaviour characteristic of *melanogaster*. The tendency of both ends to stick to the chromocentre is not affected by the presence of an extra chromosome IV from *simulans*. It is interesting to note that in triplo-IV partial hybrids the two MIV's were always found to be paired;

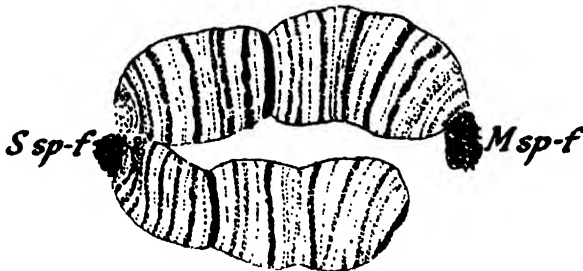


FIG. 2.—The right arms of two unpaired single chromosomes IV.

the single SIV was usually attached to the chromocentre with one end only, indicating the region of the centromere. No case of pairing of the single SIV with the two already synapsed MIV's was found in the nuclei of the salivary glands.

In diplo-IV partial hybrid with the chromosome constitution MIV/SIV, the MIV again exhibits the properties typical of *melanogaster*. The chromosome IV of *simulans* is stretched out and its one end is free, while the other is attached to the chromocentre. Occasionally the two chromosomes IV form a ring-like structure in which the centromere region of MIV lies near the free end of SIV, while the middle regions

remain widely separated. Instances were also observed when MIV and SIV lie separately stretched out and are attached at their proximal regions to the chromocentre.

In *Drosophila melanogaster* and *simulans* hybrids, Horton (1939) never found somatic synapsis of the two chromosomes IV belonging to the two species. During our study, only one salivary gland nucleus was found in which such pairing was encountered (fig. 3), a single chromosome IV of *melanogaster* being paired with that of *simulans* in the inverted middle part of the latter. It was not possible to determine exactly the limits of the synapsis and the inversion.

The absence of synapsis in ordinary hybrids and its occurrence, although rare, in partial hybrids, may probably suggest some inter-relations between the chromosomes. While in ordinary hybrids each species is represented by one chromosome of each pair, in the case of partial hybrids studied one single chromosome of one species was present in the whole chromosomal environment of the other species.

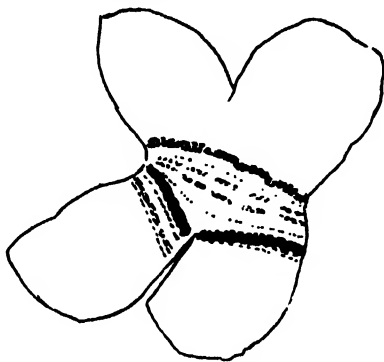


FIG. 3.—Pairing of the middle inverted parts of SIVR and MIVR.

The differences between the MIV and SIV, according to Horton, are qualitative (structural) and quantitative (numerical). There is one relatively long inversion in the chromosome IV of *simulans*: it includes about 24 bands out of the total of about 50 bands composing the whole chromosome IV of *melanogaster*. The minimum length of the inversion lies between 102 B 1-2 and 102 E 1-2. Horton considers it very probable that 102 E 3, 4, 5 bands are also included in the inversion, and our observation supports that view. The numerical change in the bands involves the "terminal ring" of the SIV, which was stated by Horton to have no corresponding structure in the chromosome IV of *melanogaster*.

Besides these differences other structural variations are also observed in the chromosome IV, namely those concerning the left arm. Griffen and Stone (1940) have proved the existence of a short IVL in *melanogaster*, and by genetical and cytological analysis were able to construct a map of this newly discovered chromosome limb. A left arm has also been identified in *Drosophila simulans* by the present author. Its structure is illustrated and the cytological map is compared with that of the chromosome IV of *melanogaster* (fig. 4); the reference system of Griffen and Stone has been adopted.

The left arm of chromosome IV is very short, measuring only 3–4 μ in the nuclei of the salivary gland, and is very thin, *i.e.* about one-third of the diameter of the other arm of IV. Furthermore, the heterochromatic material of the chromocentre usually obscures the IVL. Such structural properties and behaviour explain why it has escaped for so long the attention of cytologists. During our analysis, only 20 out of

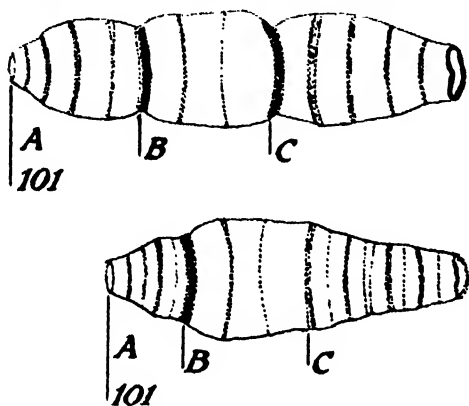


FIG. 4.—The salivary gland chromosome maps of the left arm of the chromosome IV. Upper drawing, *melanogaster*, is based on Griffen and Stone's drawings, the lower one represents a tentative map for *simulans*. The deficiency discussed in the text probably removes a middle heavy band described according to Griffen and Stone as 101 C 1 (–2); therefore the section 101 C in *simulans* begins with the band 101 C 3 of *melanogaster*.

several hundred salivary gland nuclei were encountered in which the left arm of the IV could be seen. The left arm of *simulans* and *melanogaster* IV exhibit partial synapsis; pairing is limited to the distal and proximal regions. If these arms are left unpaired MIVL forms a loop with the tip of the arm turned towards the chromocentre. The frequency of various configurations was as follows: distal and proximal synapsis was observed in 7 cases, proximal in 6, and in 7 cases they remained unpaired.

A comparison of *simulans* and *melanogaster* IVL has shown that the former is deficient in one heavy band, which is designated in *melanogaster* as band 101 C 1. It is not improbable that this particular band is composed of two elements. While partial synapsis between MIVL and SIVL has been observed in hybrids with diplo-IV constitution, no pairing between *melanogaster* and *simulans* IVL was found in hybrids having two chromosomes IV from *melanogaster* and one from *simulans*. In spite of the meagreness of our data we may attribute such failure of pairing to a lack of balance between the forces of attraction which necessarily find a different expression in a triplo-IV from that in a diplo-IV hybrid.

V. STRUCTURAL DIFFERENTIATION OF THE CHROMOSOME IV.

Heitz (1933), comparing the differentiation of mitotic chromosomes in various species of *Drosophila* into euchromatin and heterochromatin, has arrived at the conclusion that the small dot-like chromosome IV of

melanogaster is composed of euchromatic substance. Griffen and Stone (1940) support Heitz's statement. During the present study, a detailed analysis of the structure of the chromosome IV in the nuclei of salivary glands has, however, definitely shown that both ends contain undifferentiated substance, which exhibits a very close similarity to that observed in other chromosomes. Furthermore, it was found that they have a tendency to associate with the ends of other chromosomes. Such

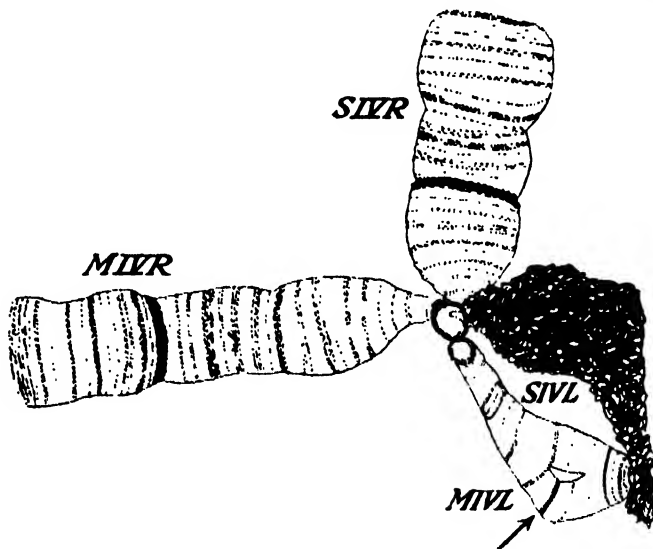


FIG. 5.—The deficiency of the band 101 C 1 (-2) in synapsed left arms of the chromosome IV of a partial hybrid containing one MIV and one SIV. The arrow indicates the band deficient in SIVL.

similarity in morphology and behaviour suggests strongly that this material represents heterochromatic regions in the chromosome IV. Besides the two distal heterochromatic regions, Panshin and Khvostova (1938) have identified the proximal inert region which is composed of heterochromatin. In view of the fact that the length of the chromosome IV during mitotic metaphase does not exceed 0.2μ , the author is of the opinion that it is hardly possible to distinguish internal differentiation of this chromosome by means of differential staining; therefore observations which are based only upon the appearance of the chromosome IV during the mitotic cycle cannot be reliable.

In *simulans* the differentiation of IV deviates from that characteristic of *melanogaster*. Horton (1939) has already drawn attention to the fact that the "terminal vesicle" or a double distal band of the right arm of SIV is absent in MIVR (fig. 2). The question now is, what is the origin

of this terminal vesicle in the SIVR? One may assume that in *simulans* the band 101 C 1 (-2) in the left arm is transferred to the tip of the right arm. In diplo-IV hybrids the left arm of MIV is bent towards the tip of SIVR and they show some kind of association at this region. Pairing between the middle region of MIVL and the "terminal vesicle" of SIVR has not been observed; in view of the extremely small size of the chromosome arms, such pairing would be expected to occur only very rarely. Though the absence of synapsis does not completely disprove the assumption that the terminal vesicle of SIVR is homologous with band 101 C 1 (-2) of MIVL, other evidence rather suggests that it corresponds to a part of the most proximal basal band of MIVR; there is a close morphological similarity between the two, and it was observed that this basal band is greatly reduced in *simulans*.

Furthermore, it was found that the tip of SIVR is always free, while that of the other chromosomes of *simulans*, as well as of *melanogaster* (including both arms of MIV), not infrequently fuse together. Such behaviour indicates that the tip of SIVR is devoid of heterochromatin. The absence of heterochromatin at the end of the right arm of IV in *simulans* opens up a very interesting problem concerning the nature of the telomere, but such a discussion is beyond the scope of this paper.

It was pointed out above that the proximal or basal band of MIV has a vesiculated structure. Bridges (1935) was the first to point out that at the very end of the basal region a vesicle or ring structure can be seen, which may be interpreted as a spindle fibre organ or as a focus of the chromocentre. Griffen and Stone (1940) regard this vesicle as the centromere. Though centromeres may be observed in mitotic and meiotic chromosomes of various organisms (Darlington, 1937), this would be the only case known in which the centromere as such is demonstrated in the chromosomes of salivary gland nuclei of *Drosophila*. Its vesiculated structure in *melanogaster* on the one hand, and the probable transference of one part of this "centromere," which does not show its centromeric character, to the tip of SIVR on the other hand, suggest that the centromere has a compound structure. In this respect the centromere of *Drosophila* would be very similar to the centromere of the chromosomes of maize (MacClintock, 1938).

During the cytological analysis of the salivary gland chromosomes it was observed that the *melanogaster* and *simulans* chromosomes IV are in association with the various major chromosomes at the heterochromatin region. In order to determine whether the association of IV with other chromosomes is at random or not, the frequency of various chromosome

associations was recorded in the salivary gland nuclei of 12 larvæ and is given in Table III.

The data clearly indicate that the frequency of association in all larvæ of different chromosomal constitution is lowest between IV and IIL, and highest between IV and IIIR. If the association of non-homologous chromosomes in the chromocentre depends on the amount of heterochromatin in the proximal region, then it is expected, on purely mechanical grounds, that the chromosomes with a large amount of heterochromatin would during the preparation break apart from the chromocentre more easily than those with a very small amount of heterochromatin. It is known that one-third of the X at mitotic metaphase is composed of heterochromatin, while the amount, though much less than in X, is about equal in the 2nd and the 3rd chromosomes. One would expect that the lowest frequency of association would be that between the chromosomes IV and X, but this is not the case. It is not improbable that there are homologous regions of varying sizes in the genetically inert heterochromatic portion of the chromosomes. It is suggested (Muller, 1940) that some chromosome regions may, as a result of duplication, become genetically inert, especially in the neighbourhood of the centromere where the crossing-over is reduced to a minimum. Homology or partial homology is responsible for the association of the proximal chromosome regions in the nuclei of salivary gland. Hence the frequency of association in the heterochromatic region may be taken as a measure of the degree of homology at this particular region between two otherwise non-homologous chromosomes.

It is interesting to note that in diplo-IV and triplo-IV partial hybrids containing an additional SIV, the frequency of association between the IV and IIIR is increased at the expense of the association between IV and X.

A comparative study of chromosome structure of *Drosophila melanogaster* and *simulans* was carried out by Kerkis (1936), Patau (1935), and by Horton (1939). Horton made extensive cytological studies of *simulans* × *melanogaster* hybrids and found 10 visible aberrations in the chromosomes of salivary gland nuclei which distinguish these two species. Besides these 10 visible differences in the morphological pattern of chromosomes, Horton found 14 regions where the synapsis is incomplete. The total number of all differences is 24, to which should be added the one observed by me in SIVL.

During the present study it was thought that it might be possible to correlate the failure of chromosome pairing in *Drosophila melanogaster* × *simulans* hybrids with the genic differentiation of these two species,

TABLE III.

No. of Larva	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.						
Constitution	MM.	MM.	MM. Total.	%	MS.	MS.	MS.	MS.	MS.	MS. Total.	%	MMS. Total.	%					
Type of association																		
IV-X	16	6	0	22	31·88	2	3	1	2	2	1	3	4	19·05				
IV-III	2	3	0	5	7·25	0	1	1	0	1	0	1	2	3	14·28			
IV-II	3	3	1	7	10·14	1	1	0	0	1	1	1	5	6·25	0	0	0	..
IV-I	7	3	3	13	18·84	1	1	2	1	3	0	7	15	18·75	1	3	4	19·05
IV-0	14	2	6	22	31·88	3	7	4	6	11	4	7	42	52·5	6	4	10	47·62
Total	42	17	10	69	99·99	7	13	8	9	18	6	19	80	100·00	9	12	21	100·01

extensive cytogenetical analysis of a great number of species hybrids having shown (*cf.* Darlington, 1932) that failure of chromosome pairing may be taken as an expression of genic differentiation.

An attempt to disclose the genic differentiation of these two species was made by Muller and Pontecorvo (1941) by employing an ingenious new method which enabled them to produce partial hybrids. The question whether their genetical findings could be explained in terms of the above cited morphological difference in salivary gland chromosomes must remain open.

The genetical analysis of partial hybrids has not proved that the IV of *simulans* needs either the SX or SIII chromosomes. In view of the fact, however, that haplo-IV *Drosophila melanogaster* are viable and fertile, as are also partial hybrids which have *melanogaster* genotype except for being heterozygous for the chromosomes IV (MIV/SIV), it would appear that the genic systems of this particular chromosome are apparently non-complementary in the two species.

The behaviour of *simulans* chromosome in the *melanogaster* genotype may indicate that besides the structural, there must be also genic differences in the constitution of this particular chromosome.

SUMMARY.

1. The author investigated the behaviour of the chromosome IV of *Drosophila simulans* in the genotype of *Drosophila melanogaster* in salivary gland chromosomes in the material received from Dr Muller. The cytological evidence shows that in the larvæ studied there was a chromosome IV of *simulans* together with one or two chromosome IV of *melanogaster* in an otherwise purely *melanogaster* nucleus.

2. A somatic synapsis has been observed between single chromosome IV of *simulans* carrying an inversion in its middle and single chromosome IV of *melanogaster*. The synapsis occurred just in the inverted region.

3. A left arm in the chromosome IV has been observed in both species separately as well as in partial hybrids. In the latter case their bases and tips do sometimes synapse. In the middle, where they do not pair, there is a deficiency in *simulans* as compared with *melanogaster*.

4. The behaviour of *melanogaster* chromosomes IV has not been changed by the presence of additional *simulans* chromosome IV. And, conversely, the *simulans* chromosome retained its behaviour unchanged. The origin of "terminal vesicle" and the lack of heterochromatin on the free end of the chromosome IV of *simulans* is discussed.

The author wishes to express his gratitude to Professor F. A. E. Crew and to Dr A. W. Greenwood for scientific hospitality at the Institute of Animal Genetics, University of Edinburgh; to Dr H. J. Muller and Dr G. Pontecorvo for kind permission to make a cytological study of their material; and to Dr P. C. Koller for his valuable help in the preparation of the manuscript.

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IX.—Cytological Analysis of Chromosome Behaviour in Three Breeds of Dogs. By **I. A. Ahmed**, B.Sc., Institute of Animal Genetics, University of Edinburgh. *Communicated by Dr P. C. KOLLER.* (With Nineteen Text-figures.)

(MS. received February 11, 1941. Read June 2, 1941.)

A CYTOLOGICAL analysis of a number of Scalyham, Spaniel, and Spaniel \times Manchester Terrier dogs was undertaken in order to determine the number and structure of chromosomes during both mitosis and meiosis in each breed, and to ascertain the differences between them in respect of chromosome number and behaviour.

1. METHOD AND TECHNIQUE.

The technique employed is described in a previous paper (Ahmed, 1940). Minouchi's A(b) fixative solution was used during the present work. Sections were cut $16\ \mu$ thick and stained with gentian violet. The drawings were made with the aid of an Abbé Camera Lucida, Zeiss 1.3 apochr. Oil immersion objective and $20\times$ or $30\times$ comp. eyepiece.

2. MITOSIS.

The spermatogonial cells could be easily recognised in the breeds studied as they have a relatively large nucleus in comparison with the other cells. During pro-metaphase, the larger chromosomes show a tendency to form groups, while the smaller members of the complement are scattered within the cell. Three more or less well-defined groups of chromosomes were identified within most of the spermatogonial cells (fig. 1). The chromosome number was found to be 78 in each breed; this was verified by the analysis of meiosis in the primary spermatocytes in which 39 bivalents were counted.

Rath (1894) was the first to report the chromosome number in the dog, which he stated to be 64. Malone (1918) found that the number was 22 in the female and 21 in the male; the latter had one sex chromosome. Minouchi (1928) counted 78 chromosomes in both sexes, and has shown that the complement of the male has a heteromorphic pair which is assumed to represent the two sex chromosomes X and Y. Our observations, therefore, verify the chromosome number given by Minouchi; in

the male of the Sealyham, Spaniel, and Spaniel \times Manchester Terrier, the diploid number was found to be invariably 78.

Owing to the great number and small size of the chromosomes in the dog it is impossible to carry out a detailed analysis of chromosome morphology during the mitotic division. However, it could be seen

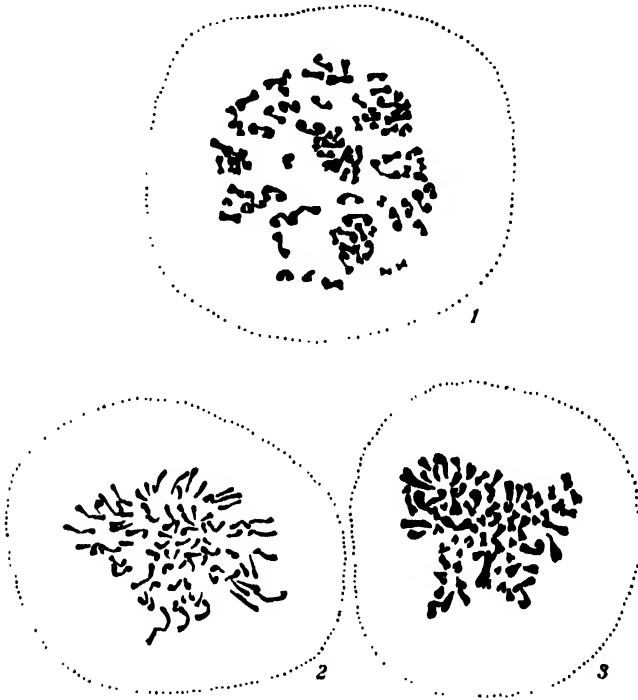


FIG. 1.—Mitotic prophase in Spaniel; the larger chromosomes form three groups.
 $\times 2550$.

FIG. 2.—Mitotic metaphase in Spaniel; the chromosomes appear to be thinner than they were at late prophase and pre-metaphase. $\times 2550$.

FIG. 3.—Mitotic metaphase in Spaniel; the chromosomes show great contraction.
 $\times 2550$.

clearly that the chromosomes are of various sizes and shapes (figs. 1, 2, and 3): the largest are about three times the size of the smallest, and there are several V-shaped ones with equal and unequal arms indicating the median or submedian position of the centromere.

It was found that later prophase and pro-metaphase chromosomes are thicker than those during the metaphase of mitosis. This appears to be a common characteristic of the chromosomes of the three types of dog studied. On the other hand, they also exhibited a few metaphase nuclei of spermatogonial cells in which the chromosomes are thicker and

shorter than in the other cells, and the distal region of chromatids of chromosomes very often lie separated (fig. 3). The structure of such chromosomes indicates that the degree of contraction is increased. The fact that cells showing such behaviour are grouped together strongly suggests that the increased rate of chromosome contraction is due to somatic mutation in the genotypic control of chromosome behaviour (Darlington, 1937).

It is evident from a comparison that the chromosomes during mitosis are very like in the different breeds investigated during the present work. No characteristics were discovered in respect of chromosome number, structure, or behaviour which could be attributed to one only of the breeds.

3. MEIOSIS.

It might be expected that if differences in chromosome behaviour occur between the breeds, they could be more easily detected by a close analysis of the meiotic division. A number of primary spermatocytes were analysed in order to determine chiasma frequencies and degree of terminalisation of chiasmata in various stages of meiosis. Table I shows the chiasma frequency at diakinesis and metaphase in the Spaniel.

TABLE I.—CHIASMA FREQUENCY IN THE SPANIEL.

Stage.	No. of Bivalents (XY excluded).	Total No. of Xta.	Total No. of Terminal Xta.	Mean No. of Xta per Nucleus.	Variance.	Mean No. of Terminal Xta per Nucleus.	Variance.
Meiotic Diakinesis.	152	323	266	80.75	5.188	66.5	1.312
Meiotic Metaphase.	380	732	670	78.2	1.760	67.00	46.600

To test for the significant difference between two means, *e.g.* between the mean number of the total chiasmata per nucleus at diakinesis and that at metaphase, the calculation made is as follows:—

$$\begin{aligned}\text{S.E. of the means} &= \pm \sqrt{5.188 + 1.760} \\ &= \pm 2.636.\end{aligned}$$

$$\text{The total chiasmata difference} = 2.55 \pm 2.64.$$

The variance of the mean number of terminal chiasmata per nucleus at metaphase in the Spaniel is apparently very large because of the great

variation among the cells concerning the number of bivalents with two chiasmata, both of them terminal or one terminal only.

The data show that in the Spaniel there is no significant difference in respect of number of chiasmata at diakinesis and metaphase. The shape and configuration of bivalents are very similar during both stages

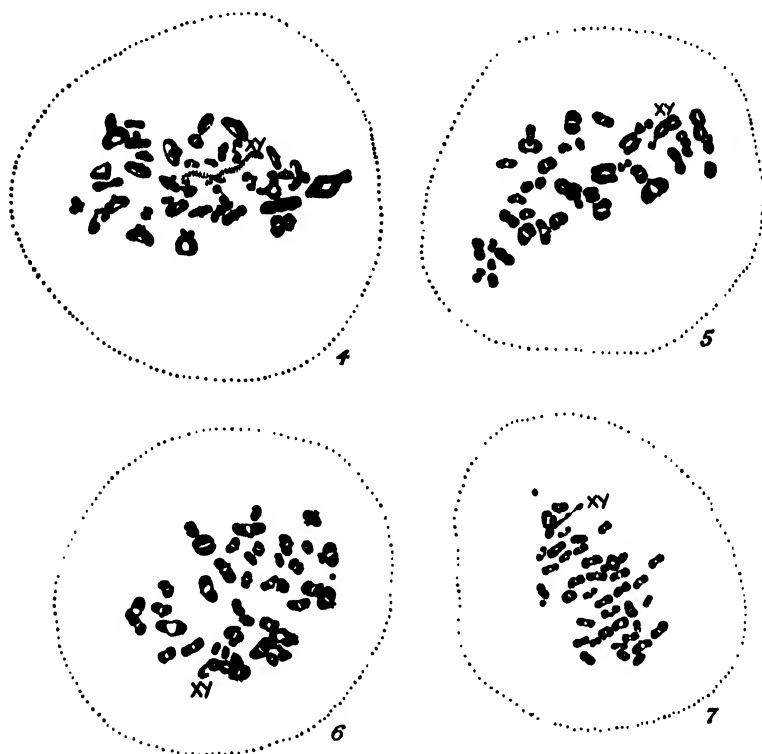


FIG. 4.—Diakinesis in Spaniel; the asymmetrical sex bivalent exhibits a lesser degree of contraction and staining than the autosomal bivalents. $\times 2550$.

FIGS. 5, 6, and 7.—Side and polar view of first meiotic metaphase in Spaniel, Spaniel \times Manchester Terrier, and Scalyham respectively. The two different types XY are indicated. $\times 2550$.

(figs. 4 and 5), which indicates that terminalisation of chiasmata is absent or too slight to be effective in reducing the number of chiasmata. The only difference which was observed between the two stages is in the degree of contraction; metaphase bivalents are shorter and thicker than those of diakinesis. Though a similar analysis of chiasma frequencies during various stages of meiosis is highly desirable in the two other breeds, laborious attempts to obtain such data were unsuccessful. This was due to the fact that in spite of extensive studies no primary spermatocytes showing the diakinesis stage were encountered in the two other breeds.

Hence, a comparison similar to that made on the Spaniel was not possible.

The analysis of metaphase bivalents proved to be an easier task, and data, collected by studying meiotic metaphases in the different breeds, are compared in Table II.





TABLE II.—CHIASMA FREQUENCIES DURING MEIOTIC METAPHASE IN THREE BREEDS.

Breed.	No. of Nuclei Examined.	Mean No. of Total Xta per Nucleus.	Variance.	Mean No. of Terminal Xta per Nucleus.	Variance.
Spaniel . . .	10	78.2	1.760	67.0	46.600
Sealyham . . .	10	82.6	13.440	72.5	5.450
Spaniel × Manchester Terrier.	10	79.3	3.294	68.6	2.240

A test of significance was carried out, and it has shown that the three breeds do not exhibit significant differences in respect of chiasma frequencies during meiotic metaphase. Though the configuration of bivalents appears to be very similar in the three breeds (figs. 5, 6, and 7), the bivalents of the Sealyham breed are smaller. The slight variations in the position of chiasmata found in bivalents at metaphase are probably due to differences in age or other environmental factors which are outside our control.

Another analysis of the structure of bivalents was made, and the frequency of various types was determined in the different breeds. Table III summarises the data obtained.

TABLE III.—THE PERCENTAGE FREQUENCY OF VARIOUS TYPES OF BIVALENTS IN THE THREE BREEDS OF DOGS DURING MEIOTIC METAPHASE.

Breed.	Type A. 	Type B. 	Type C. 	Type D. 	No. of Nuclei.
Spaniel . . .	17.63 ± 1.955	74.73 ± 2.229	7.64 ± 1.262	..	10
Sealyham . . .	9.47 ± 1.502	73.95 ± 2.252	16.31 ± 1.805	0.27	10
Spaniel × Manchester Terrier.	19.21 ± 2.024	72.10 ± 2.302	8.68 ± 1.436	..	10

The data show that although there is no significant difference in respect of the number of chiasmata per nucleus, there is a difference in respect of the distribution of chiasmata per bivalent. In the Sealyham the frequency of Type C (bivalents with 3 chiasmata, 2 of which are

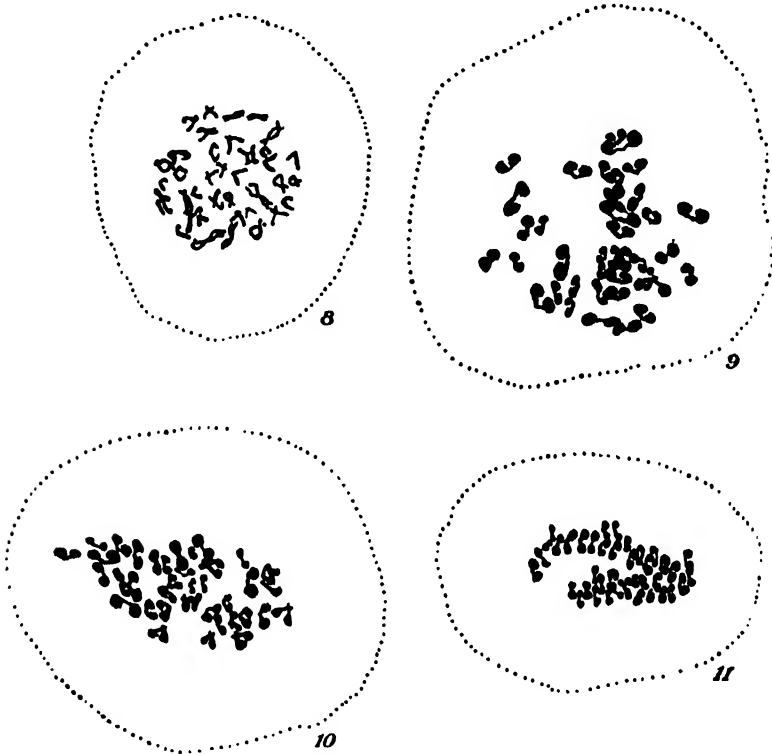


FIG. 8.—Second meiotic prophase; the chromosomes are thin and the sister chromatids are held together only at the centromere. $\times 2980$.

FIGS. 9 and 10.—Polar and side views of second meiotic metaphase. $\times 2980$.

FIG. 11.—Second meiotic metaphase, showing "hollow spindle." The chromosomes are arranged in a circle and exhibit a smaller degree of contraction. $\times 2980$.

terminal) is increased at the expense of Type A, as compared with that found in the two other breeds.

The first meiotic division in the dog is followed by a short interphase, during which the chromosomes form a more or less complete resting nucleus. At the following prophase, the chromosomes appear as thin threads, the sister chromatids widely separated and held together only at the centromere (fig. 8). This configuration is more accentuated during the following pro-metaphase and metaphase (figs. 9 and 10). Occasionally secondary spermatocytes showing "hollow spindle" during metaphase

were encountered (fig. 11). In such cells the chromosomes are arranged at the periphery of the spindle, while normally they are evenly distributed within the spindle area of the equatorial plate.

According to Darlington (1936), in cells showing "hollow spindle" repulsion of the poles may be greater at the earlier stage than in other cells, and as a result of this chromosomes are pushed to the periphery of the spindle. Another not improbable explanation is that the development of chromosomes is far ahead of that of the spindle, and the peculiar configuration of "hollow spindle" is due to this maladjustment of the time-relationship of the chromosomes and spindle.

4. IRREGULAR CHROMOSOME BEHAVIOUR DURING MEIOSIS.

It was found that in several primary spermatocytes of the three breeds a number of bivalents fail to orientate themselves in the equatorial plate during meiotic metaphase (figs. 5, 6, and 7), indicating a lack of tension between the centromeres. A similar observation was made by Darlington (1939) in various plants. It was found that non-orientation is followed by non-congression, which is due either to the crowding of bivalents on the equatorial plate, or to the fact that some of the bivalents lying outside the spindle-area are delayed in their movement towards the equator.

Frequently, in the Spaniel, primary spermatocytes were encountered in which one bivalent was lying off the equatorial plate during metaphase (fig. 12). In 16 out of 93 primary spermatocytes such a displacement of a particular bivalent was seen, suggesting that this irregularity probably arises from a definite cause. During the succeeding stage of first anaphase, lagging of one bivalent was also met with (fig. 16) and it is not improbable that the displaced bivalent at metaphase and the lagging bivalent at anaphase is the same. Such behaviour of bivalents may ultimately lead to non-disjunction of homologues.

Another irregularity encountered in the Spaniel is a lack of co-ordination between the spindle and some of the bivalents (fig. 13). Similar observations were made by Upcott (1937) in pollen-sterile *Lathyrus odoratus*.

Several primary spermatocytes were found in all three breeds in which the size of every bivalent at metaphase is apparently smaller than the size of bivalents in the adjacent cells. It was observed that the size of cells is normal, while that of the bivalents is distinctly reduced (fig. 14). It is interesting to note that while in some spermatogonial cells an increased rate in chromosome contraction was observed, during meiosis a reduction

in *size* of chromosomes was encountered. These irregularities are probably due to a change in the genotypic control responsible for determining chromosome size and the degree of contraction postulated by Darlington (1936 and 1937).

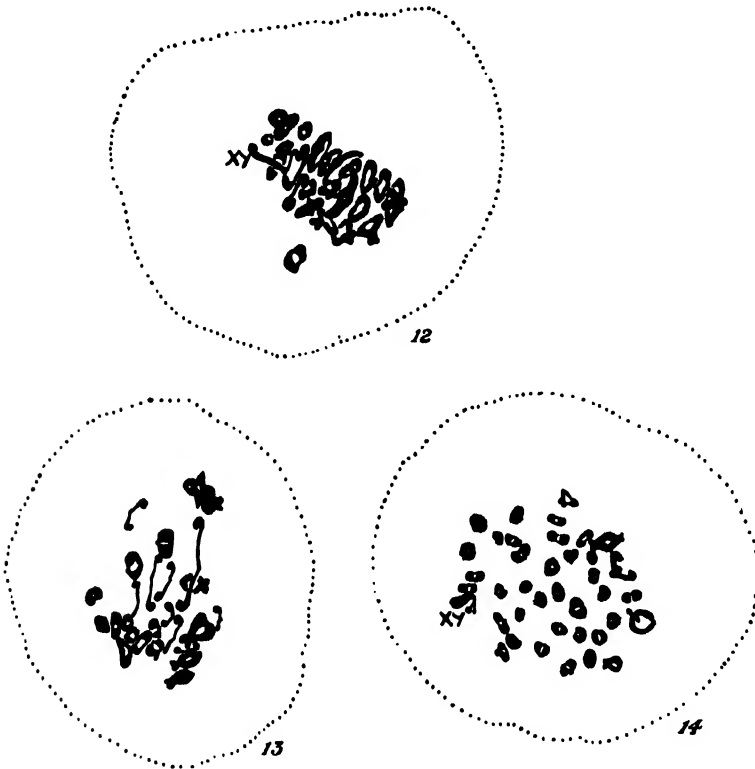


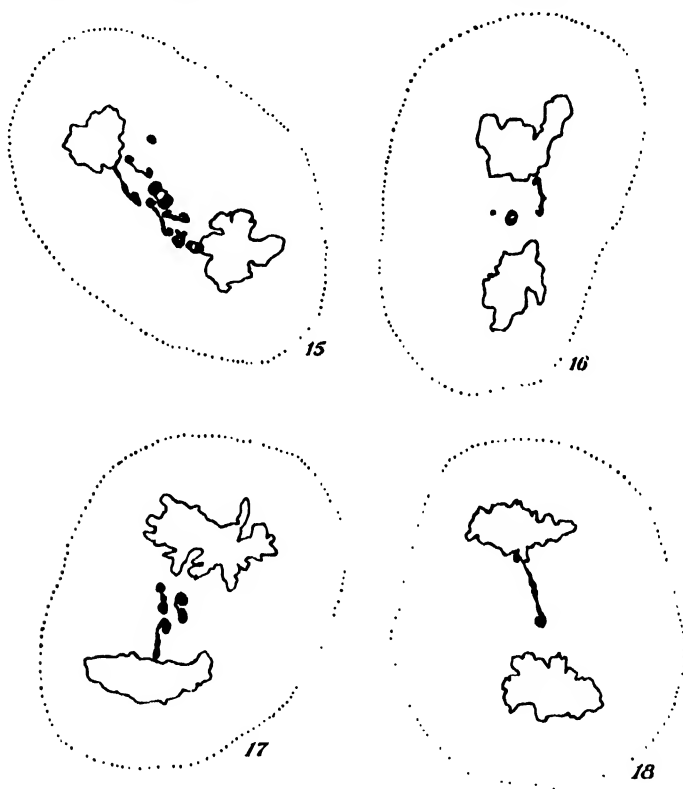
FIG. 12.—First meiotic metaphase, showing one bivalent lying off the equatorial plate. $\times 2980$.

FIG. 13.—First meiotic metaphase, showing a lack of co-ordination between the spindle and some of the bivalents. $\times 2980$.

FIG. 14.—First meiotic metaphase, in which the bivalents are of smaller size than normal. $\times 2980$.

At the first meiotic anaphase, besides lagging bivalents, thin connections between members of bivalents were observed in a few primary spermatocytes in the Spaniel (figs. 15, 16, and 17). Such configurations resemble chromatid bridges which are due to crossing-over within a relatively inverted segment of chromosomes (*cf.* Darlington, 1939). The frequency of primary spermatocytes showing "bridges" is very low, and this is to be expected in view of the fact that cells showing first meiotic anaphase stage are very rare, indicating that this stage is a very

short one. Furthermore, the great number of bivalents, and their small size, makes it very difficult to detect real chromosome or chromatid bridges, and it is not improbable that we are dealing with a delayed separation of bivalents instead of bridges due to inversion. The presence of a chromatoid body (figs. 6, 7, 15, and 16) in the primary spermatocytes



FIGS. 15-18.—First meiotic anaphase illustrating lagging of bivalents. $\times 2550$.

can also easily be mistaken for a fragment which results from single crossing-over within the inversion. Our data, owing to difficulties enumerated above, are not critical enough to state with certainty that the Spaniel studied was heterozygous for an inversion.

5. THE STRUCTURE OF SEX CHROMOSOMES.

It was mentioned above that during mitotic metaphase one heteromorphic chromosome pair is present in the diploid complement of those breeds investigated. Our observation verified that made by Minouchi (1928), according to whom the male has an XY sex-determining

mechanism, but was contrary to the view of Malone (1918), who believed that the male sex in the dog is of the XO type, having only one sex chromosome. In the present study it was definitely established that there are two sex chromosomes present which differ in size; the larger is designated the X, while the smaller is called the Y chromosome.

At the pachytene stage of meiosis, the two sex chromosomes are associated at one end (fig. 19, *a*), and it is assumed that they are held together by one subterminal chiasma (*cf.* Koller and Darlington, 1934). It can be seen that the behaviour of XY is precocious as compared with

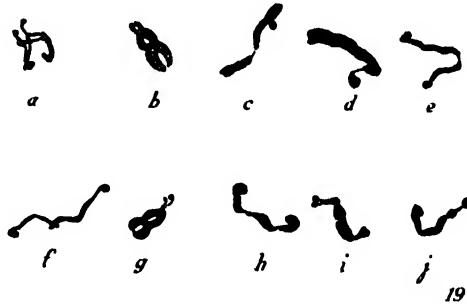


FIG. 19.—The different configurations of XY bivalent during the different stages of meiotic division. $\times 3800$.

that of the autosomes. During later stages of meiosis, when the structure of XY complex could be seen more clearly, a higher number of chiasmata was occasionally observed (fig. 19, *b*, *g*). It was found that the development of the sex bivalent precedes that of the autosomal bivalents. The shape and configuration of the XY complex is very variable, indicating the presence of regional differences in the internal organisation of these particular chromosomes. There is a portion of the X and Y which, during the prophase of meiosis, remains in a diffuse stage for a long period. The centromere region of one sex chromosome is often attenuated and therefore appears as a thin thread: two terminal knobs of varying sizes, which are widely separated, could be seen frequently at the end of the long thin thread (fig. 19, *d*, *i*, and *j*). Such a configuration may be misleading, for the thin thread could easily be interpreted as a terminal chiasma connecting the two unequal sex chromosomes at metaphase. It is interesting to note that during diakinesis stage the sex bivalents are much thinner and longer than during previous stages of meiotic prophase (fig. 19, *e* and *f*). The XY complex exhibits a less degree of staining at diakinesis (fig. 4) than at metaphase.

At meiotic metaphase two types, the asymmetrical and symmetrical XY bivalents, were observed in the various breeds (figs. 5, 6, 7, 12, 14,

19, *g*, *h*, *i*, and *j*). The asymmetrical type was identified by Minouchi (1928). The presence of the two types during meiosis suggests that the sex chromosomes have a centromere which is localised in the pairing or homologous segment of X and Y: consequently chiasmata could be formed at either side of it (*cf.* Koller and Darlington, 1934; and Ahmed, 1940). Owing to the difficulty of analysing individual bivalents at the meiotic metaphase it was not possible to obtain sufficient data to enable me to determine the frequency of these types in the different breeds. At the first meiotic anaphase lagging of the XY pair was also encountered (fig. 18). The structure of this lagging bivalent has clearly shown that the X and Y chromosomes differ in respect of size.

The author wishes to acknowledge his indebtedness to Professor F. A. E. Crew and Dr A. W. Greenwood for their encouragement and hospitality, to Dr P. C. Koller, under whose guidance the work was carried out, and to the Royal (Dick) Veterinary College, Edinburgh, and the Genetical Society of Britain for generously supplying the material.

SUMMARY.

1. The diploid chromosome number in the Spaniel, Sealyham, and Spaniel \times Manchester Terrier is 78. The chromosomes differ in size and shape.
2. No characteristics were found in respect of chromosome number, structure, or behaviour during mitosis which could be attributed to one only of the breeds.
3. Chiasma frequency in the Spaniel is about the same during diakinesis and metaphase of meiosis.
4. Among the three breeds no significant differences were found in respect of chiasma frequencies per nucleus during meiotic metaphase.
5. In the Sealyham the frequency of bivalents with three chiasmata is increased at the expense of bivalents with two chiasmata, as compared with the two other breeds.
6. Non-orientation, non-congression, and lagging of bivalents were found in all the breeds.
7. The sex-determining mechanism consists of a heteromorphic chromosome pair. At meiotic metaphase the asymmetrical and symmetrical XY bivalents were observed in the various breeds. It is assumed that the centromere is localised in the pairing segment.

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(Issued separately August 8, 1941.)

X.—Geological Notes on the Stubendorff Mountains, West Spitsbergen. By **W. B. Harland**, Gonville and Caius College, Cambridge. *Communicated by* Dr G. W. TYRRELL. (With Three Plates and One Text-figure.)

(MS. received October 28, 1940. Revised MS. received February 25, 1941.
Read January 13, 1941.)

CONTENTS.

	PAGE		PAGE
I. INTRODUCTORY	119	V. SUBSEQUENT HISTORY	126
II. GEOLOGICAL INTRODUCTION	120	(a) Cretaceous and Tertiary Events	126
III. THE METAMORPHIC ROCKS.	121	(b) Evolution of Present Topography	127
(a) Historical	121	(c) Present Erosion	127
(b) Petrography	121	VI. SUMMARY AND CONCLUSION	128
(c) Stratigraphy	122	VII. REFERENCES TO LITERATURE	128
(d) Tectonics	123	VIII. DESCRIPTION OF PLATES	129
IV. THE CARBONIFEROUS ROCKS	125		

I. INTRODUCTORY.

THE Cambridge Spitsbergen Expedition, 1938 (*Polar Record* No. 17, p. 4), was led by L. H. McCabe for intensive geomorphological work in the Campbell Range at the head of Billen Bay, Ice Fjord. The principal work on nivation and corrie erosion (McCabe, 1939) was supplemented by topographical and geological survey, while some detailed investigations were made on the raised beaches and "soil polygons." During this work a party of three crossed the ice divide by sledge to East Fjord, Wijde Bay, and spent ten days working from the northern side of the Stubendorff Glacier. The Stubendorff Mountains in this region were chosen for comparison of corrie erosion on account of their contrasting geological constitution. They are carved out of tough folded metamorphic rocks while the Campbell Range is formed of softer horizontal Carboniferous rocks.

The time for effective survey in this area was greatly diminished by mists around the mountain-tops. M. B. Adams, who undertook a compass traverse, was thus hampered. A sound topographical basis has since proved necessary for an interpretation of the geological structure from my records, and as the results of the Oxford University Arctic Expedition, 1933, coastal survey of Wijde Bay (Glen, 1934) and the

Norwegian Government aerial survey of 1936 (Hoel, 1937) and 1938 have not been available, much of the subsequent work has consisted in producing a map consistent with the compass traverse, the aerial photographs, the geological records, and other photographs. This has been effected by trial and error through adjusting a plasticine model so as to utilise fully the available evidence by visibility checks. The map (Pl. III, *a*) has been constructed from the model (Pl. I, fig. 1), which was flooded in stages to indicate the form lines, and the geological structure was plotted on a plaster cast. A cement cast showing the extent of the ice to a scale of 1 inch to 1 mile is now deposited in the Scott Polar Research Institute, Cambridge. The papers referred to include most of the relevant maps.

II. GEOLOGICAL INTRODUCTION.*

The Stubendorff Range, with its continuation along the west coast of New Friesland and inland to the east and south, consists of rocks which have mostly undergone high-grade regional metamorphism and have been deformed so as to exhibit a great variety of structure. The dips are usually high, and the strikes trend north and south. Overlying these schists and gneisses is occasionally preserved the remains of an old peneplain on which rest almost horizontal Carboniferous rocks. This most conspicuous and regular unconformity dips in a south-westerly direction, so that the southern component of one degree brings Carboniferous rocks from the summit of the coastal Stubendorff Mountains down to sea-level just north of the Campbell Range. To the east, in the centre of New Friesland, still higher mountains are not capped by Carboniferous.

Wijde Bay, of which East Fjord is the southern branch, follows the line of a fault separating the structure of New Friesland from the Devonian mountains on the west, which were not visited.

New Friesland supports a covering of "highland" ice, and this flows out along a number of steep-sided valleys through the coastal ranges.

It was only in a comparatively small area of the coastal region that the following notes were made, and thus, while more intensive work was possible than on previous sledge traverses, it remains exploratory in character and raises more questions than it solves.

* The geology of Spitsbergen was conveniently summarised by Frébold (1935), though he had not incorporated the latest information regarding the geology of New Friesland.

III. THE METAMORPHIC ROCKS.

(a) *Historical.*

These rocks have been variously termed the "Basement Complex," "Urgebirge," "Western Schists and Gneisses," and "Hecla Hoek Formation." Here they are shown to be at least pre-Upper Carboniferous, and elsewhere similar rocks are pre-Downtonian. Apart from this upper limit, however, little is known regarding their age. It is generally accepted that Caledonian diastrophism accounted for their tectonics and metamorphism just as it is responsible for the less disturbed Hecla Hoek rocks to the east.

The Hecla Hoek formation is composed of an immense thickness of sediments ranging at least from Lower Cambrian to Lower Ordovician; their general correlation with the rest of Spitsbergen and elsewhere is fully discussed by Kulling (1934).

There is now a tendency to regard the western schists and gneisses as the equivalents of the Hecla Hoek. This view is developed by Odell (1927) and confirmed by Fleming and Edmonds (1934), each of whom have sledged past the Stubendorffs. They conclude that the western schists and gneisses are the higher grades of regional metamorphism of the same series, and these lower zones have been uplifted and exposed to the west and pass eastwards into the zones of lower grade. A hypothetical line of discontinuity was previously introduced because so much of the transition is obscured by ice. No attempt is made here to correlate in detail the paragneisses with the Hecla Hoek sediments, but their petrography suggests that correlation is feasible if in fact the same series are represented.

The rocks correspond with many of Tyrrell's (1922) descriptions of morainic boulders to the south, and sections to the north, of the Norden-skiöld Glacier, and the tectonics appear to be similar. More recently Fairbairn (1933) has described minutely the petrography of rocks more nearly associated with the present area. He accompanied the Cambridge Spitsbergen Expedition, 1932, which sledged to the east of the coastal range, and he describes additional specimens collected by the Cambridge Spitsbergen Expedition, 1930, under the same leadership (Jackson, 1931), and this expedition visited the Stubendorff Glacier.

(b) *Petrography.*

The rocks yield most attractive specimens and slices, which show that they have been wholly recrystallised under stress to form schists, granulites,

and gneisses. I have passed my specimens on to Edmonds and Fleming to help complete their work on the relations between the various Hecla Hoek rocks in the whole of New Friesland, as the detailed petrology is more relevant to their study than mine. High-grade stress minerals are characteristic, and micas, hornblende, and garnet are major rock-forming minerals. The following determinations are of frequent rock-types used in mapping, though they may form an inadequate petrological basis for classification; for instance, I may well have confused ortho- and para-gneiss in the abundant pink felspathic quartz-granulites:—

Orthogneiss:

Basic—Amphibolites, including garnet-amphibolites, hornblende-schists, and hornblende-plagioclase-gneiss.

Acid—Granitic gneiss, including pink augen-gneiss and hornblende-biotite-gneiss.

Paragneiss:

Psammitic—White and coloured quartzites and schist.

Pink felspathic quartz-granulites.

Mauve foliated quartz-muscovite-(felspar-garnet)-gneiss with white quartz lenses.

Pelitic—Brown garnet-biotite-schists.

Calcareous—White forsterite-marble.

(c) *Stratigraphy.*

I hazard under this heading some notes on the grouping of these rock-types, but in no case was the order of superposition directly determined. The groups are lettered for convenience in reference only (text-fig. 1).

A. The amphibolites are the most widespread rocks and occur in many associations. Most conspicuous is the pink- and black-banded formation, in which thick wavy gneiss up to 200 feet thick is interspersed with thinner pink felspathic quartz-granulites. This was probably formed by recurring flows of basalt intercalated in sediments.

B. Another group of rocks which constitute an apparently conformable series contains at least 1000 feet of mauve psammitic rocks associated with thinner developments of pelitic schists and marbles. The mauve rocks are the foliated quartz-muscovite-schists with felspar and garnets and white quartz lenticles.

With a steep dip and well-developed joint planes they weather out as tough grey slabs which dominate the two superb coastal corries.

The garnetiferous biotite-schists are reddish brown in appearance, with bands of siliceous nodules giving a yellowish coloration.

The marbles are well jointed, and there are at least three horizons of the order of 50, 200, and 400 feet. They form conspicuous white features in the cliffs though not resistant to weathering.

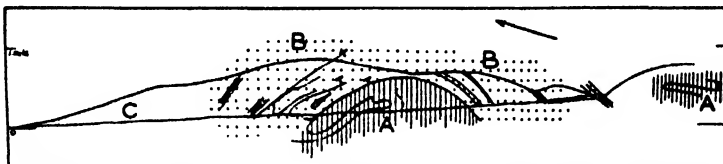


FIG. 1.—Diagram suggesting partial interpretation of Caledonian tectonics.

A, B, and C refer to tentative stratigraphical groups explained in the text, Section III (c).

This group contains little amphibolite, and where it does occur there are often developments of granitic gneiss.

C. The rocks of the coastal area lack cliff exposures and it is difficult to determine their relations.

They are predominantly psammitic schists with numerous variations, from white quartz schists to pelitic schists. Amphibolite intrusions are frequent, either as massive irregular invasions associated with local folding and displacement, or as thin, regular, and "stepped" sills in quartzite.

(d) *Tectonics.*

In such a region the studies of stratigraphy and tectonics are mutually dependent, but time permitted detailed study of neither. It is hoped that the following structural observations will compensate for this, although it was not found possible to make detailed measurements of successions. The observations are conveniently classed in two groups. When the mist obscured the heights, the coastal roches moutonnées, beach platforms, and corries were examined, and when the light was favourable the mountains and cliff faces were viewed from a distance. The former intensive work is analogous to mapping in (say) Anglesey, which demands prolonged work with an accurate topographical map, while the latter finds its counterpart in the Alps, where the key to the structure may be obtained rapidly from commanding positions.

I will first enumerate the more detailed observations and then view the structure from a distance.

The strike of each formation was remarkably constant, and altogether conformed to the general direction between N. and N.N.W.

The dips varied greatly and, while in many areas the beds were folded, the predominant dip in the west was westerly and in the east easterly, with a common value of about 50° .

Joint planes often controlled the cliff faces, and their dominant strike was approximately perpendicular to that of the beds.

Foliation and cleavage had the same strike as the beds, and in the coastal corries the "flow cleavage" was almost perpendicular, while what may have been a "fracture cleavage" dipped about 20° to the west as compared with the bedding dip of 50° to 70° . The few records are consistent with the tectonic interpretation, but they were only measured in one corrie.

Isoclinal folding was often suspected and was photographed near the coast (Pl. II, fig. 1), and disharmonic folding was often observed on both a small and large scale. The small-scale folding was similar to the larger structures later postulated. Mica schists function as incompetent beds and zones of thrusting, while the most competent appear to have been the marbles and foliated psammitic rocks. While most resistant to denudation, the amphibolites have behaved as though folded in a plastic condition, whether they occur in the more regular flows or as irregular lenticular masses and *lit-par-lit* injections in Group C (Pl. II, fig. 2).

The marbles and mica schists seem to be the key horizons which would most repay further mapping. Both occur as conspicuous bands on account of their colour and less resistant weathering, and while the marbles are identifiable by their thickness, which should remain constant, being competent beds, the schists are characterised by yellow siliceous bands. Both are valuable as an index of metamorphic grade.

Stepping back from the exposures and viewing the cliff faces along the strike from the south (see composite section, Pl. III, *b*), one receives a general impression of recumbent folding and thrusting with nappe structure (text-fig. 1). Overfolding to the west has undoubtedly taken place, and what first appeared as an anticline must be a culmination (text-fig. 1). The core of this (Group A) may be an older series, and this structural unit extends along the same strike to outcrop in the sides and nunataks of the Mittag-Leffler Glacier ten miles to the south. The overlying beds (Group B) show more tendency to thrusting and cleavage than to forming the smooth flowing lines of the underlying recumbent folds. At least one small nappe was seen (Pl. I, fig. 2). Moreover, I think many of the rocks from the coastal corries (Group B), and possibly even to the coast (Group C), can best be interpreted as the overthrust or overfolded portions of the easterly dipping rocks to the east. Other

interpretations involving involutions with higher or lower structural units may be possible, such that Group C is related to Group A or independent, but further speculation is unprofitable.

Rocks of Group A were again seen in the most easterly exposures, and these too were overfolded, with anticlines apparently rising westwards, as though here were roots of the higher folds or nappes.

All these structures conformed to the geosynclinal rather than the foundation type of folding, and no trace of a rigid block was found.

In considering the history of the pre-Carboniferous events successive stages may be distinguished, but all appear to be part of a single orogenesis. During the deposition of the sediments there was periodic eruption of basic magma. This was followed by folding, possibly with further intrusions of both basic and acidic composition, and thrusting. Elsewhere large acid intrusions occur at a late stage in the tectonic events (Odell, 1927). The "culmination" may be due either to the thrusting or "flowing" of nappes and folds over a block, now submerged, or to subsequent arching (text-fig. 1). Such arching may have accompanied the granitic intrusions of Mt. Newton and Mt. Chernishev in the vicinity. The hypothesis of arching has the same effect as Odell's conclusions regarding a differential elevation about some hinge-line. There is also agreement in interpreting overthrusting to the west in the Alpine sense, but it is meaningless to discuss further from which side the pressure came. Odell suspected isoclinal folding in the Hecla Hoek rocks of central and eastern New Friesland, and in correlating this area with Hinlopen Strait rocks, Kulling (1934) requires even more modification of the central succession on account of repetition and thrusting. The present area is of interest in exhibiting these structures more clearly, and especially in the deeper rocks, where deformation has resulted in flow rather than fracture.

It is assumed that these changes took place in Silurian time prior to the deposition of Downtonian sediments in the north-west, and are related to the Caledonian movements in Europe and Greenland. At least there was time subsequently for denudation to reduce some thousands of feet of crystalline rocks to the pre-Carboniferous peneplain.

IV. THE CARBONIFEROUS ROCKS.

On the above-mentioned erosion surface rests a series of gently dipping sandstones and shales. The rocks peep out at a few points from beneath the ice carapace on the flat tops of the coastal mountains, and it is clear that these truncated mountains owe their form to the removal of softer rocks from this unconformity.

Along the coast a fault, seen in the sides of the corries, has a downthrow to the west, increasing at an angle of 5° to the south as compared with the slight dip of the main peneplain. On this side about three hundred feet of the series were hurriedly examined and found to consist of a deltaic facies similar to the Yorkshire Estuarine Series. They were mostly dark shales with interbedded sandstones and ganisters. At the base of the series was a conglomerate consisting of quartz pebbles, grains, and cement, with occasional finely crystalline darker rocks. Higher up, beds of sideritic ironstone and ferruginous micaceous sandstone were conspicuous in the scree. The sandstones were variously spotted, false-bedded, or with ripple marks or shale pellets. Sometimes thicker lenses suggested sand-filled channels. The shales and mudstones, often slicken-sided, graded into small impure coal seams.

Carbonised plant remains occurred frequently. Sandstone casts of *Lepidodendron* were found on either side of the fault and other forms were collected—all tree roots or stems. Dr E. Dix reported that these were not well enough preserved for specific identification.

Carboniferous rocks have previously been recorded from the east and south of New Friesland, but their facies (deltaic, gypsiferous, dolomitic, lagoonal, and coral) are too variable for distant correlations to be attempted on lithological grounds.

V. SUBSEQUENT HISTORY.

(a) *Cretaceous and Tertiary Events.*

No deposits since the Carboniferous rocks are preserved in this area except those formed recently.

An east-west dolerite dyke was seen at the base of the Mittag-Leffler Glacier just to the south of the area mapped. This is probably one of the large group of Neocomian dolerite intrusions recorded from most other regions in Spitsbergen (Tyrrell and Sandford, 1933).

The nature of the fault running N.W. across the map was not discovered. The N.S. fault seen in the coastal corries is determined by the underlying strike of the metamorphic rocks, as is also the main faulting responsible for Wijde Bay. This is probably part of the widespread Tertiary block faulting, when the warping of the Carboniferous peneplain may also have taken place. The corrie fault follows a band of granitic gneiss, and fault breccia of contorted Carboniferous sandstone and gneiss has been formed.

(b) Evolution of Present Topography.

The form of the present surface is mainly due to structural control, which can now be appreciated. In addition to the major effects of block faulting the region is a dissected plateau the remnants of which are picked out by ice carapaces.

The southern face of the mountain is controlled by jointing, and the continuity of beds in direct alignment to the south of the Stubendorff Glacier suggests that there has been no faulting. The two coastal corries have cut back into the foliated gneiss with walls defined by joint planes, and at their mouths the faulted Carboniferous rocks form low scree-covered bluffs. These rest on a floor of massive amphibolite which determines the level of the corrie mouth. The other corries follow the strike, and two are in line with major bands of mica schists.

The most peculiar elongated ridges and troughs along the coastal belt follow the strike constantly, and in some cases the surfaces of these rounded ridges follow the bedding planes of anticlines and in others the structures have been truncated by ice erosion.

In this coastal belt ice and sea have left evidence of recent physiological conditions.

From the extensive *roches moutonnées* and from the moraines it is clear that not only did the ice extend far beyond its present position, but that even recently the ice has been and is retreating in the corries and glaciers.

A series of raised beach platforms to the height of at least 400 feet form the coastal lowlands. This stage succeeded the glacial occupation, as the *roches moutonnées* of the ridges pass underneath beach deposits in the troughs (Pl. II, fig. 1). Elsewhere in Spitsbergen, and notably in Ice Fjord, numerous tilted raised beach deposits of similar height occur (Tyrrell, 1922, pp. 41-43). Following on the last great ice extension these raised features indicate isostatic recovery of some magnitude.

On the raised beach deposits, and also on the Carboniferous scree slopes, solifluction has taken place, with the formation of a lateral soil profile and polygonal surface markings which are such a prominent feature of the Campbell Range. However, with these exceptions, pedogenic processes are at a minimum, and the surface is old and stable so that vegetation has become established in the hollows while moss and lichens often cover the boulders in the lowlands.

(c) Present Erosion.

Erosion at the present day is mainly due to the action of running water. As the glaciers retreat leaving terminal and lateral moraines,

these are being demolished by the sea and by the streams which are extending the fluvio-glacial plains.

McCabe (1939) showed how ice is principally effective in corrie erosion through the short-period freezing and re-thawing of melt-water running behind the ice, and a similar process is taking place on a smaller scale in nivation, where the comminuted debris is washed away by trickling water.

VI. SUMMARY AND CONCLUSION.

The principal results of the work are summarised on the map and sections (text-fig. 1 and Pl. III). Much has been left unsolved, but the indications of overfolding to the west and intense Caledonian metamorphism fulfil the expectation of previous workers, and Carboniferous rocks are first recorded here.

In conclusion, I would say that we went from the Campbell Range to the Stubendorff Mountains for geomorphological contrast and our journey was abundantly justified. It was only possible through the planning and leadership of McCabe and through the co-operation of the other members of the expedition. When considering the foundations of the expedition in Britain and Norway it is difficult to know where indebtedness ceases—whether it be for kindness and consideration, or the necessities of finance, equipment, and food. However, I do wish to record my special gratitude to Dr Tyrrell for his constant help.

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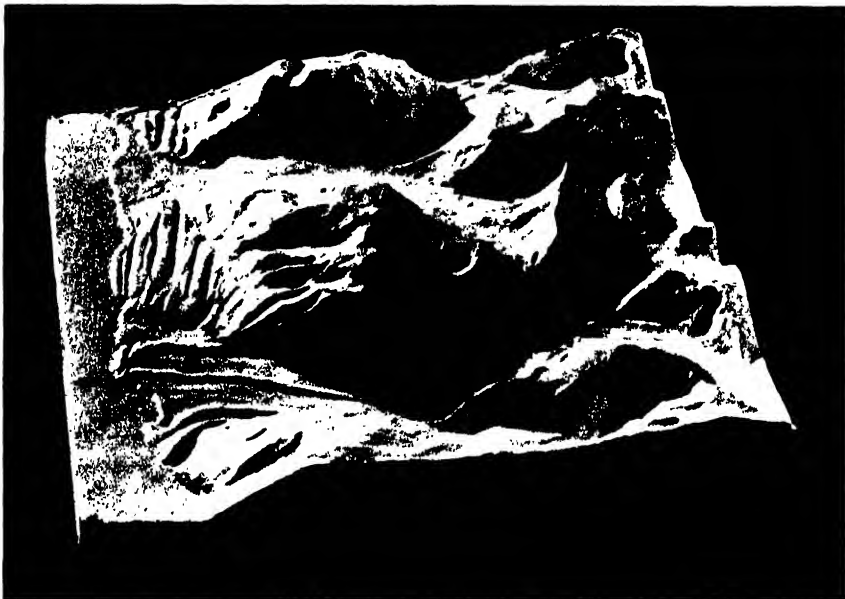


FIG. 1



FIG. 2



FIG. 1

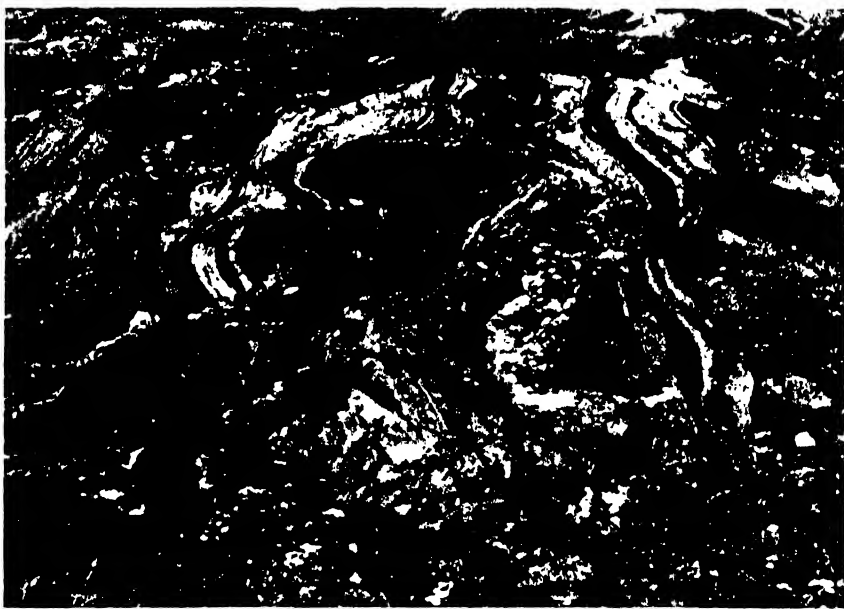
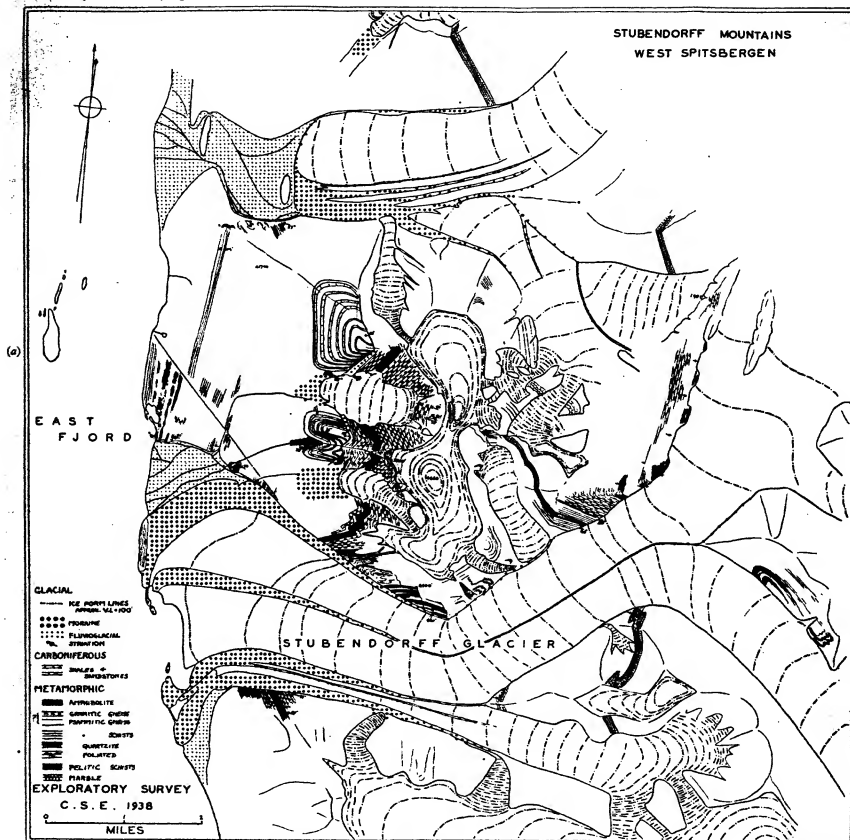


FIG. 2



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VIII. DESCRIPTION OF PLATES.

PLATE I.

Fig. 1. Plaster cast of model—view from S.S.W.

Fig. 2. Southern face of mountain viewed from medial moraine of Stubendorff Glacier. On the right is the supposed recumbent syncline in rocks of Group A, and on the left is the marble core of the small nappe in Group B.

PLATE II.

Fig. 1. Isoclinal folding in pelitic and psammitic gneiss. Striations on the roches moutonnées may be seen. Beach deposits occupy the foreground, and the Devonian mountains are on the far side of Wijde Bay.

Fig. 2. Amphibolite intrusions in psammitic gneiss.

PLATE III.

(a) Geological map of the area described. Topographical frame adjusted from various data. Peripheral region less reliable.

(b) Projection of some features on to plane perpendicular to the dominant strike. Natural scale.

KEY TO GEOLOGICAL SYMBOLS.

Glacial: Broken form lines of ice, approximate V.I.=100 feet.

Moraine—stippled with spots.

Fluvio-glacial—stippled with fine dots.

Carboniferous: Thin-jointed bands on white background.

Metamorphic: Details on p. 122.

Amphibolite—black; granitic gneiss— $\times \wedge \vee$; pink granulites—white bands associated with amphibolites; quartzites—parallel lines with dots; mauve foliated rocks—parallel lines with sigmoid curves; Biotite-schists—close parallel lines; marble—parallel lines with joints.

XI.—On the Sphincter Valve of the Antennal Gland of *Marinogammarus marinus* (Leach) sens. str. By Mary V. Schorstein, B.Sc. (Lond.). From the Department of Zoology, Victoria University, Manchester. *Communicated by* Professor H. GRAHAM CANNON, F.R.S. (With Four Text-figures.)

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Read July 7, 1941.)

INTRODUCTION.

THE sphincter valve between the end sac and the duct in the antennal gland of *Gammarus pulex* was first described by Vejdovsky (1901) as consisting of "three cells forming a funnel-apparatus surrounded by a ring muscle." According to this author "one can convince oneself of the muscular cell producing the fibrils of the muscular ring" (p. 382). Zavadsky (1914) confirmed the presence of fibrils, but failed to find a muscular cell apart from the valve cells, and concluded that "the muscle ring appears as a differentiation of the nephridial wall" (p. 97). Recent work by Cannon (1923, 1925, 1926, 1931, 1940) and by Cannon and Manton (1927) indicated that fibrils found in connection with a valve are invariably situated within the valve cells.

The present work was undertaken in order to ascertain the exact relation of the fibrils to the cells of the valve in Gammarids. An attempt was also made to determine the nature of the fibrils, and to obtain information about the derivation of the valve cells. Both *Gammarus pulex* and *Marinogammarus marinus* were examined, and although the following description refers specifically to the latter, it applies equally to *Gammarus pulex*, for the structure of the sphincter valve appears to be identical in the two species.

The present investigation was undertaken at the suggestion and under the direction of Professor H. G. Cannon. I wish to express my thanks for his help and advice.

METHODS.

The material was fixed in Duboscq-Brasil and embedded in paraffin wax. Transverse, parasagittal, frontal and oblique sections 6μ , occasionally 8μ , in thickness were cut, and were stained either with Mallory's

triple stain or Heidenhain's iron hæmatoxylin. Borax carmine, alum carmine, orcein, Verhoeff's elastic tissue stain and Weigert's resorcin fuchsin were also employed.

Vejdovsky (1901, p. 382) states that in his preparations the myofibrils were stained "an intense red" with carmine. The present author has

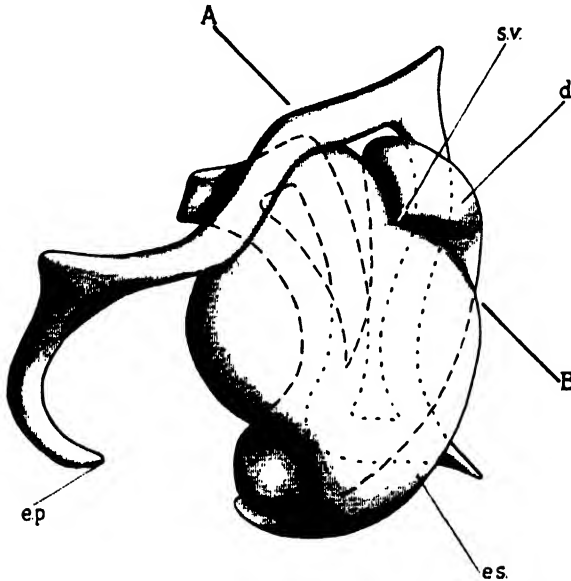


FIG. 1.—Reconstruction of Left Antennal Gland from left anterior aspect. ($\times 150$ approx.) d., first loop of duct; e.p., excretory pore; e.s., end sac; s.v., position of sphincter valve.

Line AB, plane of sections shown in fig. 3.

been unable to obtain anything except a very faint stain—quite useless for critical work—with both borax and alum carmine.

DESCRIPTION.

The general anatomy of the gland was first described by Leydig (1860) and later by Sars (1867). More detailed descriptions of its anatomy and histology were given by Grobben (1880) and Vejdovsky (1901). Little need be added to their observations, but it may be mentioned that the gland in the adult stage of *Marinogammarus* (fig. 1) shows an additional loop as compared with Grobben's illustration (fig. 4, Taf. 1) of the gland in a young specimen. Contrary to Vejdovsky's statements, in the walls of the duct no cell membranes separating the nuclei can be distinguished. There appear to be about 50 nuclei in the walls of the duct.

The sphincter valve lies in the most lateral region of the base of the antenna at the junction of the duct with the end sac (fig. 1). It consists of six cells (fig. 4) of which three, the anterior sphincter cells, are actually part of the end sac wall. They form the sides of an anterior triangle surrounding the exit from the end sac. Alternating with the anterior cells are three posterior sphincter cells, whose anterior portions form the walls of the tube connecting the end sac with the duct, whilst their posterior, pear-shaped portions bulge into the lumen of the duct.

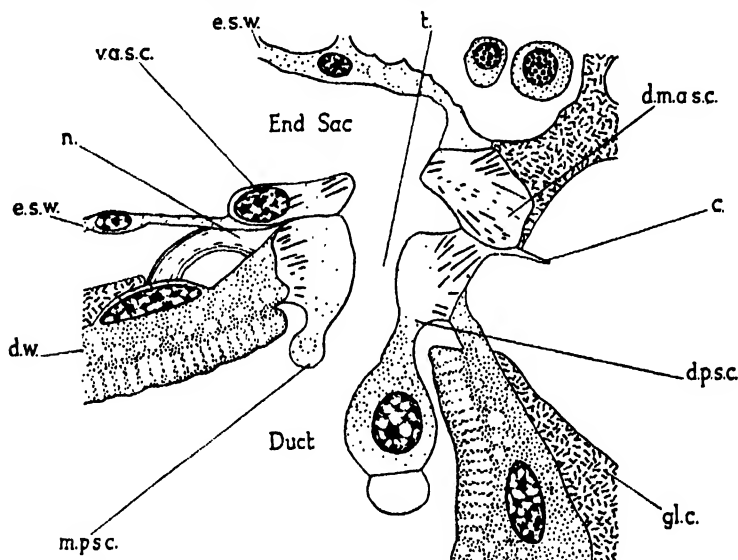


FIG. 2.—Slightly oblique parasagittal section through the sphincter valve of the Left Antennal Gland. ($\times 1333$ approx.) c., connection with cuticle; d.m.a.s.c., dorso-medial anterior sphincter cell; d.p.s.c., dorsal posterior sphincter cell; d.w., wall of duct; e.s.w., wall of end sac; g.l.c., glandular cell; m.p.s.c., medial posterior sphincter cell; n., nerve; v.a.s.c., ventral anterior sphincter cell; t., tube leading from the end sac to the duct.

The posterior sphincter cells correspond with Vejdovsky's "funnel cells" (1901, p. 381, Pls. 26, 27; figs. 13, 14, 18), and their respective positions are dorsal, medial, and ventro-lateral. The anterior portion or pedestal of each cell appears approximately pentagonal in transverse section. The outermost corners of the pedestals of the dorsal and ventro-lateral cells are connected with the cuticle by coarse strands of connective tissue (fig. 3, b). One corner of the pedestal of the medial cell is joined by connective tissue to a coil of the duct which in its turn is firmly held by tonofibrils to the cuticle of the mid-ventral region. The pear-shaped portions of these posterior sphincter cells consist each one of a massive vesicular nucleus surrounded by finely granular cytoplasm,

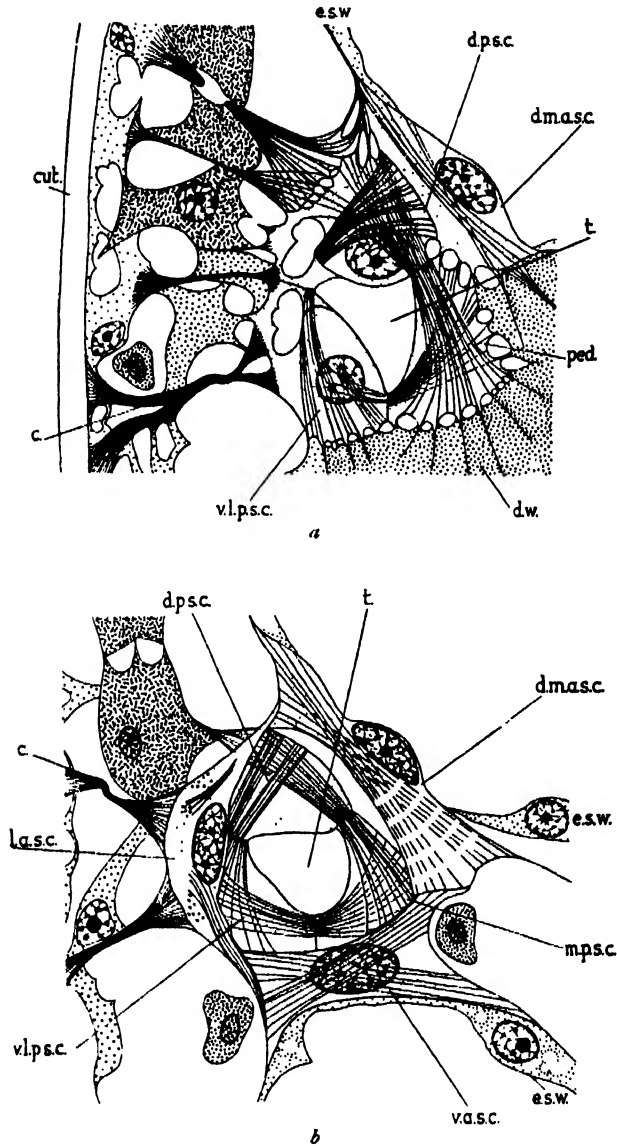


FIG. 3.—Series of two transverse sections through the sphincter valve of the Right Antennal Gland; (a) through the pedestals of the posterior sphincter cells along the line AB shown in fig. 1; (b) through the anterior sphincter cells. ($\times 1000$ approx.) c., connection with cuticle; cut., cuticle; d.m.a.s.c., dorso-medial anterior sphincter cell; d.p.s.c., dorsal posterior sphincter cell; d.w., wall of duct; e.s.w., wall of end sac; l.a.s.c., lateral anterior sphincter cell; m.p.s.c., medial posterior sphincter cell; ped., pedestal of medial posterior sphincter cell; v.a.s.c., ventral anterior sphincter cell; v.l.p.s.c., ventro-lateral posterior sphincter cell; t., tube leading from the end sac to the duct.

from which, as in *Gigantocypris* (Cannon, 1940), hang large droplets of secretion about to be shed into the duct (fig. 2).

The anterior sphincter cells occupy dorso-medial, lateral, and ventral positions respectively (fig. 3, *b*). Only one of these was observed by Vejdovsky who, assuming that it gave rise to all the fibrils of the "muscular ring," named it "the muscular cell" (1901, pp. 382, 383, Pl. 26, fig. 14). He also pointed out that, particularly in *Niphargus*, the valve cells protrude "into the coelomic cavity like lips" figs. 2, 3,

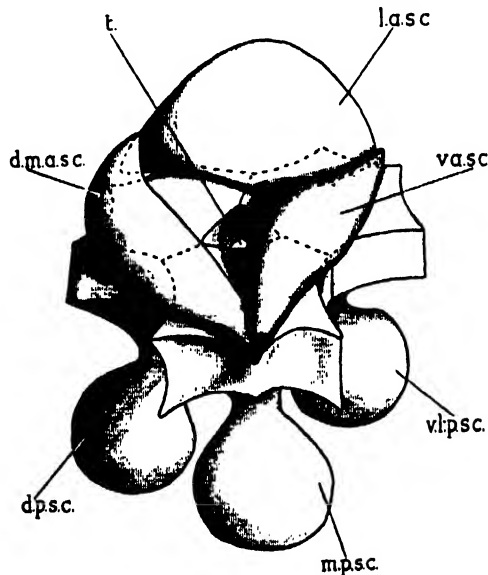


FIG. 4.—Reconstruction of Left Sphincter Valve from medial aspect. ($\times 750$ approx.) d.m.a.s.c., dorso-medial anterior sphincter cell; d.p.s.c., dorsal posterior sphincter cell; l.a.s.c., lateral anterior sphincter cell; m.p.s.c., medial posterior sphincter cell; v.a.s.c., ventral anterior sphincter cell; v.l.p.s.c., ventro-lateral posterior sphincter cell; t., tube leading from the end sac to the duct.

12, 13, 17, 18); and it seems probable that these bulges are, in fact, cells of an anterior tier. The dorso-medial and ventral cells of this tier are roughly spindle-shaped. The lateral anterior sphincter cell is disc-shaped and completes the triangle. Its situation in the lateral wall of the end sac is such that its greatest area appears in parasagittal section, whilst in transverse section it is seen as a narrow strip. Coarse fibrils of connective tissue attach the cell to the cuticle. The cytoplasm of these cells is thin and vacuolated in the marginal regions where it joins the neighbouring cells of the end sac.

The six valve cells have been referred to as sphincter cells because each contains myofibrils. In the clear cytoplasm of the "pedestal" of

each posterior sphincter cell, the myofibrils are arranged in two sets which cross each other. Each set extends diagonally across the pedestal, and in transverse section (fig. 3, *a*) is seen to spread fanwise from one edge of junction with a neighbouring pedestal to the opposite outer edge.

Two sets of fibrils may also be observed in each cell of the anterior tier. Each set spreads from the point of junction with an adjacent anterior sphincter cell outwards and anteriorly to the edge which merges into the end sac wall. About the centre of the cell, the two sets of fibrils cross.

The fibrils are highly refractile, and react to stains in the same manner as skeletal muscle. They exhibit a negative reaction to such elastic tissue stains as orcein, Verhoeff's stain, and Weigert's resorcin fuchsin, but, what is more important, in several specimens the fibrils showed transverse striations comparable with the Q discs and Z lines (Krause's membrane) of relaxed skeletal muscle (fig. 3, *b*).

In one preparation there were indications of striated fibrils in all six valve cells. In others, various cells of both anterior and posterior tiers contained such fibrils. It was noticeable that when striated fibrils were found in valve cells of the left antennal gland, they were also to be found in those on the right side of the body.

DISCUSSION.

The morphological findings described in this paper are at variance with Vejdovsky's account of the "funnel-apparatus" (Trichterapparat) as consisting of three valve cells surrounded by a "ring muscle" produced by a special muscular cell. The present investigation leads to the conclusion that six cells all containing myofibrils constitute the valve.

Vejdovsky assumed that the valve cells projecting into the end sac of *Niphargus* were derived from its lining of coelomic epithelium. Cannon and Manton (1927, p. 445), however, demonstrated that valves having a similar appearance may originate either from the mesoderm, as in the maxillary gland of *Chirocephalus*, or from the ectoderm, as in the maxillary gland of *Cyprinotus* (Cannon, 1925). The posterior sphincter cells of *Gammarus* are connected with the cuticle in a manner comparable with that seen in *Cypris*, and it appears probable that at least these cells are ectodermal in origin. That such ectodermal cells may produce typical striated muscle has already been demonstrated by Cannon (1926, 1931, 1940) and Manton (1928). The derivation of the anterior sphincter cells is more doubtful; for whilst the lateral anterior

cell is connected with the cuticle in a manner comparable with the ectodermal sphincter cells of a freshwater Cyprid, thus suggesting its possible ectodermal origin, yet the fact that these three cells form part of the wall of the end sac points rather to a mesodermal origin.

The fibrils were stated by Vejdovsky to consist of "smooth muscle throughout" (p. 383), and have generally been assumed to be muscular in nature, although Ter-Poghossian (1909) suggested that they might be elastic. Their staining reactions, and the frequent presence of typical cross-striation, as described in the present paper, leave little room for doubt that a close relationship exists between the fibrils of the valve and the myofibrils of skeletal muscle. The fact that the fibrils were striated in some preparations and not in others may indicate degrees of functional activity as shown by Carey (1921), and the difference between striated and unstriated muscle in this instance may well be conceived of in terms of physiological activity, rather than rigid anatomical classification.

The arrangement of the fibrils within the cells differs from any so far described. In the posterior sphincter cells, it is comparable to that in the sphincter cells of *Gigantocypris* (Cannon, 1940), if one imagines the tetrahedral arrangement there found to be compressed into one plane. It is, however, possible to comprehend the variability in the arrangement of fibrils within the various types of sphincter cell by taking the view that wherever cytoplasm is subjected to tension, there arise, along the line of stress, fibrils which tend to become striated if the tension be maintained.

SUMMARY.

1. The sphincter valve of the antennal gland of *Marinogammarus marinus* consists of six cells arranged in two tiers each containing three.
2. Each cell contains two sets of myofibrils which sometimes exhibit transverse striation.

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(Issued separately October 23, 1941.)

XII.—The Avian Ulna: Its Quill-Knobs. By **George H. Edington, M.D., D.Sc., and Agnes E. Miller, M.A.,** Glasgow.
Communicated by Professor E. HINDLE, M.A., Sc.D., Ph.D
(With Eight Plates.)

(MS. received February 15, 1940. Revised MS. received May 1, 1941.

Read May 5, 1941.)

INTRODUCTION.

THE presence upon the ulna of many birds of a row of smaller or greater tuberosities, their variety in size and conformation, and the presence of other appearances on the surface of the ulna suggested that an investigation of these variations and of the development of the structures might yield points of interest.

Owen (1848) was apparently the first (we have been unable to consult Brandt's paper, quoted by Gadow) to draw attention to these ulnar markings, related to the quills of the secondary remiges. In that year he published a schematic outline drawing of the avian forelimb. Neither in this nor in a subsequent publication (1849), however, did he describe the linear series of markings on the ulna shown in the drawing; but in 1866 he stated that "on the ulnar and anconal sides of the shaft are the rows of quill-knobs (in *Raptores*) for the secondaries; the anconal row is most marked in longipennate *Natatores*, and is the only row in many birds." He mentioned also that this character is wanting in the flightless and some other birds. Pl. I, fig. 1, will be seen to correspond closely with his description.

Prior to Owen, Vicq d'Azyr (1805), Blumenbach (1807), Tiedemann (1810), and Meckel (1829) do not mention any markings, and Tiedemann merely states that the hinder wing feathers lie on the forearm. Of writers subsequent to Owen, Huxley (1879) describes the ulna as often presenting "a series of tubercles indicating the attachment of the secondary quill-feathers." Gadow (1891) describes the arm feathers as resting on the ulna and producing upon the dorsal surface many small roughnesses, which he called "*Exostoses*." Pycraft found along the postaxial border of the ulna of various birds a "row of small tubercles" for the attachment of the secondary remiges; and in 1907 he describes them as "prominent tubercles." Steiner (1918) refers to the "sogenannten ulnaren *Exostosen*"

(p. 300) in many birds, and describes their appearance. Pelissier (1923) in an article on the ligamentary apparatus of the remiges deals with the musculo-tendinous structures of the forearm and their bearing on the movements of the quills. He mentions the *ligament cubital* which fixes both the remex and its satellite *teatrice* to the tuberosities of the ulna. He considers that the ligament serves as a hinge for the remex in all its movements on the forearm. He preserves, however, a noticeable silence with regard to the tubercles of the palmar row. Stresemann (1934), in much the same terms as Gadow (*supra*), writes that the arm-wings fasten themselves to the ulna, thereby producing exostoses on the dorsal surface of the bone. There is no mention made of the lower, or palmar, series of knobs.

The present communication comprises a description of these structures in a limited number of examples, a description of the ligamentous attachments between them and the secondary remiges, and some histological observations on the development of the quill-knobs. In making the drawings from museum specimens special attention has been paid to bringing out the variations in the *form* of the quill-knobs. Certain of these ulnæ and knobs are figured in Meyer's Atlas (1879), but without any description. Some of the specimens figured by us are not represented in that work in corresponding detail, while others do not appear at all.

We should like to acknowledge the advice and help of Professor Hindle of the Department of Zoology, University of Glasgow, to whom also we are indebted for laboratory facilities, and we owe much to Professor James Ritchie, Edinburgh, for many helpful suggestions. Our thanks are also offered to Mr J. Fleming for facilities at the Kelvin-grove Museum, to Miss Brown Kelly for the fidelity to detail of her beautiful drawings, to Dr M'L. Watson, Physiology Department, University of Glasgow, for interpretation of certain histological appearances, and to Mr A. Mackinnon for the preparation of sections. Reproduction of the illustrations has been generously assisted by a grant from the Carnegie Trust for the Universities of Scotland, to whom we express our cordial thanks.

THE QUILL-KNOBS OF CERTAIN AVIAN ULNÆ.

There is present upon the ulna of *Pelecanus onocrotalus* ♀ (Pl. I, fig. 2) an anconal row of twenty quill-knobs in the form of rounded tubercles, distinct at the proximal end of the bone and more flattened in shape towards the distal end. In addition, on the surface of the bone

nearer to the radius a series of wavy markings, almost hollows, is apparent. The anconal knobs of *Ciconia alba* ♀ (Pl. I, fig. 3) form a row of small distinct spines almost uniform in size except for one or two in the middle of the series, which are linear in shape. The appearances in the male (Pl. I, fig. 4) differ noticeably from those in the female. In the former towards the carpal end of the ulna the anconal series is represented by depressions or pits (four in number) from the floor of which arise small protrusions. The series is then continued proximally by blunt spines, followed in their turn by less obvious elevations. Several shallow depressions finish the series. In addition, four small distinct elevations are to be seen above, *i.e.* nearer the radius than the anconal row. Moderately sharp spinoids characterise the anconal row of the quill-knobs of *Platalea leucorodia* ♂ (Pl. II, fig. 5), the distal members tending to be elongated and ridge-like, contrasting with the proximal which are more rounded. In this bone the series is not quite regular, as one elevation at the carpal end is more pronounced and is separated from its immediate neighbour by a distinct gap. In the ulna of *Casarca rutila* ♀ (Pl. II, fig. 6) a series of linear elevations is present with the proximal members more pronounced than those nearer the carpal end of the bone.

The quill-knobs of the Condor, *Sarcorhampus gryphus* ♂ (Pl. II, fig. 7), form a series of prominent rounded projections with flat and slightly hollowed tops. In contrast to this appearance the anconal series in *Serpentarius secretarius* (Pl. III, fig. 8) is in the form of thickenings of a continuous ridge and it is noticeable that the knobs at the carpal end differ from those at the humeral end, the former being more linear and the latter more rounded in shape. This appearance of the quill-knobs as thickenings of a continuous ridge is still more pronounced in the ulna of *Neophron percnopterus* ♂ (Pl. III, fig. 10) with the proximal four elevations standing out distinctly. In *Aquila clanga* ♂ (Pl. IV, fig. 11) the quill-knobs appear as very faint elevations, the terminal knob at the humeral end being a definite tubercle. On the curved ulna of *Falco jugger* ♀ (Pl. IV, fig. 12) this linear character of the knobs is more pronounced than in any other we have examined, but while in this case the proximal knob is very faintly marked, the succeeding three elevations alter in appearance and assume a spinoid form. Wavy markings above the quill-knobs, as in the Pelican (Pl. I, fig. 2), are to be observed in two cases, *Serpentarius* (Pl. III, fig. 8) and *Falco* (Pl. IV, fig. 12). To this we refer later.

Antigone australasiana ♀ (Pl. III, fig. 9) shows an anconal row of uniformly shaped spines extending along the entire length of the bone.

From the angle at which the drawing has been made, and also because of their proximity to each other, both anconal and palmar rows of spines can be seen. Comparing this specimen with a male example (Pl. IV, fig. 13), the anconal knobs here are in the form of tubercles rather than spines, are less distinctly marked, and the more distal members have almost disappeared. In this drawing the palmar series is just visible. Well-marked tubercles characterise the anconal knobs of the ulna of *Balearica pavonina* ♂ (Pl. V, fig. 14).

The quill-knobs of *Numenius arquata* ♂ (Pl. V, fig. 15) are in the form of somewhat faintly marked linear elevations resembling, but rather more pronounced than, the quill-knobs of *Aquila* (Pl. IV, fig. 11). In *Larus ridibundus* (Pl. V, fig. 16) the elevations are in the form of tubercles possessing ridges; in this drawing both rows of knobs are seen to lie parallel to each other, and the palmar knobs are similar in form to but smaller than the anconal. *Corvus frugilegus* (Pl. VI, fig. 18 (1)) exhibits an anconal row of six knobs in the form of tubercles. These are well-marked at the carpal end of the bone (Pl. VI, fig. 18 (1) C), while at the proximal end of the series the terminal knob is much less pronounced, resembling a scar rather than an elevation. The palmar row comprises four knobs, the most distal being obviously a tubercle and the remainder becoming more linear and less distinct. The two rows may be seen to converge towards the humeral end of the bone, a contrast with the more strictly parallel arrangement in the black-headed gull (Pl. V, fig. 16).

In interesting contrast to these outgrowths on the ulna of many birds is the presence on the ulna of *Spheniscus Humboldi* (Pl. VI, fig. 17) of two rows of small *pits* on the anconal surface.

ADDITIONAL ULNAR MARKINGS.

Additional markings are present on the anconal surface of the ulna of several of the specimens figured. These markings are situated nearer to the radius than the anconal row of knobs, and are shown in six of the figures. In five of these (Pl. I, fig. 2, Pl. II, fig. 6, Pl. III, figs. 8 and 9, and Pl. IV, fig. 12) they take the form of faint lines which curve and cross the long axis of the bone. With one exception the lines start from respective quill-knobs. The general effect is produced of slight hollowing of the areas of bone enclosed by the lines. Their significance is not clear: possibly they form *points d'appui* for the proximal ends of the quills in certain positions of the wings. When present, the appearance of the bone is suggestive of rippled sand uncovered by a falling tide. The

sixth figure is an example of something quite different, viz. a supernumerary row of anconal knobs (Pl. I, fig. 4).

In the female *Pelecanus* (Pl. I, fig. 2) the lines form almost semi-circular curves convex towards the radius. They start from the respective knobs and form a series of "pot-hooks," of which the points stop well short of the next distal knob.

In *Casarca* (Pl. II, fig. 6) faintly elevated but obvious lines are visible, starting from the middle of each anconal knob and running obliquely and distally towards the radius.

These linear markings are also curved in *Serpentarius* (Pl. III, fig. 8), with convexity proximal and, arising from the knobs as in *Casarca*, they end by pointing towards the carpal end of the bone.

In the Juggler Falcon (Pl. IV, fig. 12) the lines arise *between the knobs* and are curved or widely angular with convexity distal. The general appearance is that of a series of chevrons crossing the long axis of the bone.

The female *Antigone* (Pl. III, fig. 9) shows very faint linear markings, which start from the individual knobs and cross the ulna obliquely in a proximal direction.

The sixth (exceptional) example is found in the male *Ciconia* (Pl. I, fig. 4) already referred to, in which there occurs a *supernumerary row* of four knobs.

ANATOMICAL RELATIONSHIPS OF ULNAR KNOBS.

Dissections were undertaken to investigate the relationship between the "quill-knobs" and the secondary remiges in *Columba livia*, *Larus ridibundus*, and *Corvus frugilegus*. With regard to the last-named, all the specimens were in apparently adult plumage. The birds, however, were sexually immature, a *bursa Fabricii* being present in every case save one, and in that the genital tissue was very slightly developed.

In *Columba livia* exposure of the extensor aspect of the fore-arm showed the roots of the quills of the secondary remiges abutting on the anconal surface of the ulna, to which they were attached by fibrous ligamentous structures.

The quill-follicles, with which covert quills were in close relationship, were isolated. The remiges could be very fully flexed, and when brought up into the extended position they were found very quickly to lock with the subjacent ulna. The apices of the follicles were firmly adherent to the bone, the point of attachment of each being a projecting quill-knob of the anconal series.

Similarly, dissection of the extensor aspect of the fore-arm in *Larus*

ridibundus showed that when the remex was flexed towards the body of the bird the ligament, attaching the deep end of the follicle and enclosed quill root to the bone, became taut. On extending the remex upwards away from the body the ligament became relaxed and the root of the quill locked on the ulna. This "locking" was apparently brought about by the follicle, some distance up from its apex, being anchored by fibrous tissue to the palmar knob region of the bone. The rocking movements of flexion and extension of the quills took place on the subjacent edge of the ulna, and were limited by the check-action of the anconal and palmar ligaments respectively.

Dissection of the *flexor* aspect of the forearm of *Corvus frugilegus* was made for inspection of the palmar knob region. On retracting the lower lip of the incision a series of stout thread-like ligaments was demonstrated (Pl. VI, fig. 18 (2) *vl.*) passing from the deep surface of the dermis to the flexor (palmar) surface of the ulna. It was evident that each of the secondary quills (*q.*) was attached by one of the ligaments extending from the quill-follicle, some way up from the apex, to the surface of the ulna. As shown in Pl. VI, fig. 18 (2) the ligaments are more compact or cord-like towards the carpal end of the bone (C) than those at the humeral end, where they are thinner and more spread out as they pass on to the bone.

The other forearm was dissected from the extensor (anconal) surface and the fibrous connexion between the quills and the anconal knobs exposed. The attachments were severed, and the limb then opened on the ventral surface, when it was found that the palmar ligamentous threads had been damaged. This suggested that the fibrous connexions to the two sets of knobs were not distinct from, but to some extent continuous with, one another.

To ascertain further the relationship between anconal and palmar ligaments another dissection was made. A portion of the ulna (Pl. VI, fig. 18 (3) *u.*) was isolated with the remex, third from the carpal end, in its follicle (*q.*) and the covert feather (*c.*) lying immediately above. The connective tissue attachment between the quill and the bone is displayed with differentiation of the fibres into anconal (*d!*.) and palmar (*vl.*) ligaments respectively.

HISTOLOGY.

The histological investigation of the connexions between the secondary remiges and the ulna were studied in *Corvus* and *Larus*.

Corvus frugilegus.—The rook embryo investigated was slightly more advanced than the 10-day stage of the fowl embryo. The digits and

claws of the hind limb were developed. Feathers in appearance like coarse hairs were to be seen in a row on either side of the vertebral column, also in the tail region. A few were present across the thighs, and a conspicuous row along the outer surface of the forearm.

In a transverse section (Pl. VII, fig. 19) through the forearm at this stage a lattice-work of bone (*b*) is seen to be in process of formation around the cartilaginous element. The periosteum is well defined, and at one point issuing from it a strand of connective tissue (*c.t.*) is seen stretching towards, though not quite reaching, the quill-feather rudiment (*f*). Examination of serial sections shows a succession of similar strands along the length of the ulna.

Larus ridibundus.—Paraffin serial sections 15 μ thick were cut through the forearm of a nestling black-headed gull. The projecting portions of the secondary remiges in this particular specimen averaged a quarter of an inch long, and the feather rudiment was still completely enclosed in its sheath. Pl. VII, fig. 20, depicts the appearances in transverse section through the ulna. A portion only of the ulna is shown, but the connective tissue attachment (*c.t.*) from ulna to quill feather (*f*.) is seen in its entirety. Ossification is taking place in the ulna, and osteoblasts (*ost.*) are seen also under the periosteum. In following the sections serially it was found that this connective tissue attachment between the ulna and the quill-follicle extended throughout twelve sections, *i.e.* was approximately 180 μ thick. Investigations along the series of sections demonstrated a succession of such attachments.

Similar transverse sections were made through the limb of a black-headed gull at a slightly older stage, *i.e.* running about and using its wings to assist progress. In these sections the elements already described above may be recognised (Pl. VIII, fig. 21). The bony tissue (*b.*) of the ulna has increased in extent and the periosteum (*p.*) enclosing an area of osteoblasts is also more pronounced. The connective tissue attachment (*c.t.*) has greatly increased, so that within the limits of one section only portions are present. That portion of the structure continuous with the periosteum is seen to be cut longitudinally, whilst beyond it the attachment is cut transversely. The tissue is manifestly ligamentous, though in the area* in Pl. VIII, fig. 21 and also in fig. 22, which represents a portion of the same section at a higher magnification, there are certain cells suggestive of *cartilage* at an early stage of development. As in the case of the younger gull, these ligamentous structures form a succession along the length of the ulna.

DISCUSSION.

As we have already mentioned, numerous bird ulnæ are figured in Meyer's Atlas and on many of these the quill-knobs are represented. *Ciconia alba* (Meyer, Pl. CXIX) shows the knobs in as clear detail as our illustration. *Pelecanus onocrotalus* (Meyer, Pl. CXXXI), *Balearica pavonina* (Meyer, Pl. CVI) and *Corvus frugilegus* (Meyer, Pl. XCVI) fail to show quill-knobs with the clearness of detail displayed by our drawings: the rest of our figures are either not represented in that work, or if so the quill-knobs are not shown.

A consideration of some of the ulnæ figured in this paper demonstrates that much variation of the quill-knobs may be found within the limits of a single order. The character of the knobs in the Pelican (Pl. I, fig. 2) may be contrasted with that exhibited by the Spoonbill (Pl. II, fig. 5); or, less obviously, the fainter quill-knobs in the Curlew (Pl. V, fig. 15) with the more distinct elements in the Gull (Pl. V., fig. 16); or again, the prominent quill-knobs of the Condor (Pl. II, fig. 7) with the considerably fainter series in the Spotted Eagle (Pl. IV, fig. 11). Belonging to this last order we have examined the ulnæ of a merlin (*Aesalon regulus*) in which the quill-knobs are merely faintly marked scars.

It is stated (Steiner, 1918, p. 300) that the prominence or otherwise of the quill-knobs and the strength or weakness of the ligamentous connexions of the remiges to the ulna varies inversely with the degree of development of the metapatagial muscle. This is an interesting correlation which we are unable to explain—the study of the metapatagial muscle was not included in the present investigation. The statement, with regard to the dimensions of the quill-knob varying directly with the strength or weakness of the ligaments, would seem to imply that an increased strain is thrown on the ligaments when the muscle is poorly developed, and *vice versa*.

The depressions or pits on the ulna of *Spheniscus Humboldi* (Pl. VI, fig. 17) resemble those which have been noted in the case of *Spheniscus demersus* and *Eudyptes minor* by Steiner, who regards them as "no other than exostoses" (p. 310). It is suggested that these may indicate a hereditary tendency in the penguin ulna to reproduce prominences, which tendency, owing to the special adaptation of the wing, has been checked in the course of development, resulting in a modification which appears to be characteristic of the bone in the group.

In addition we draw attention to certain markings on the extensor surface of the ulna, alongside the knobs, which are not mentioned in any of the literature we have seen, and a suggestion is made as to their

significance. Dissections show that ligaments attach the quill to both rows of knobs on the ulna and that these ligaments are to some extent continuous with one another.

Sections through the forearm show that in all the early stages of development the one element which is consistent in origin and appearance is the connective tissue attachment between the ulna and the quill-follicle. Even in the embryo rook where there can as yet be no movement of the feathers there is this distinct and regularly appearing thickening of the connective tissue surrounding the ulna and stretching towards the region of the quill rudiment. Up to the oldest stage of nestling gull examined by us there is no sign of osseous thickening of the ulna in the region of the attachment. In that stage there are present in the substance of the connective tissue attachment itself certain cells strongly suggestive of cartilage cells at an early stage of development. These might indicate the basis for the future quill-knobs. Evidence that the knobs appear late in development is afforded by the case of a rook in apparently adult plumage but which had not yet begun to fly. When both ulnæ were macerated the bones were found to be perfectly smooth, without a trace of quill-knobs.

SUMMARY.

1. The size and shape of the quill-knobs on the ulna of birds of the same order show considerable variation.
2. Eighteen specimens are figured, the salient features compared and discussed.
3. Attention is called to the presence of additional ulnar markings which have not been previously described.
4. Dissections showed that each quill is attached to the ulna by two ligaments, the dorsal being fused to the anconal knob and the ventral to the palmar knob.
5. Histological examination demonstrated the nature of the ligamentous bands connecting the quill-follicle with the periosteum of the ulna.

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DESCRIPTION OF PLATES.

All the figures reduced to $\frac{2}{3}$ size of original drawings.

PLATE I.

- Fig. 1.—Left ulna, probably of Lesser Black-backed Gull, *Larus fuscus*. *a*, "anconal" knobs alone; *b*, bone turned upwards to show "ulnar" knobs.
- Fig. 2.—White Pelican, *Pelecanus onocrotalus* ♀. Additional markings across extensor surface of ulna form a series of "pot-hooks."
- Fig. 3.—White Stork, *Ciconia alba* ♀.
- Fig. 4.—White Stork, *Ciconia alba* ♂. Supernumerary row of four knobs seen in mid-ulnar region.

PLATE II.

- Fig. 5.—Spoonbill, *Platalea leucorodia* ♂.
- Fig. 6.—Ruddy Sheldrake, *Casarca rutila* ♀. Additional markings are in form of parallel oblique lines.
- Fig. 7.—Condor "Vulture," *Sarcorhampus gryphus* ♂.

PLATE III.

- Fig. 8.—Secretary "Vulture," *Serpentarius secretarius*. Series of additional hook-like markings pointing towards carpal end of ulna.
- Fig. 9.—Eastern Sarus Crane, *Antigone australasiana* ♀. Additional markings, very faint, pass from knobs proximally and obliquely.
- Fig. 10.—Egyptian Vulture, *Neophron percnopterus* ♂.

PLATE IV.

Fig. 11.—Spotted Eagle, *Aquila clanga* ♂.

Fig. 12.—Jugger Falcon, *Falco jugger* ♀. Additional markings form a series of chevrons across the ulna.

Fig. 13.—Eastern Sarus Crane, *Antigone australasiana* ♂.

PLATE V.

Fig. 14.—Crowned Crane, *Balearica pavonina* ♂.

Fig. 15.—Common Curlew, *Numenius arquata* ♂.

Fig. 16.—Gull, *Larus ridibundus*. Both rows of knobs are seen.

PLATE VI.

Fig. 17.—Penguin, *Spheniscus Humboldi*, with quill-knobs replaced by double row of pits.

Fig. 18.—Rook, *Corvus frugilegus*. (1) Ulna, macerated, shows both rows of knobs, converging slightly as humeral end is approached. (2) Dissection of flexor surface of forearm displays ventral ligaments passing up to "ulnar" knobs. (3) Dissection to show dorsal and ventral ligaments from quill sheath to bone. C, carpal end of ulna; c., covert quill; dl., dorsal ligament; q., quill; u., ulna; vl., ventral ligament.

PLATE VII.

Fig. 19.—Transverse section through forearm of *Corvus frugilegus* at state slightly later than 10-day Fowl embryo. b., bony tissue of ulna; c.t., connective tissue advancing from periosteum (p.) to f., feather rudiment.

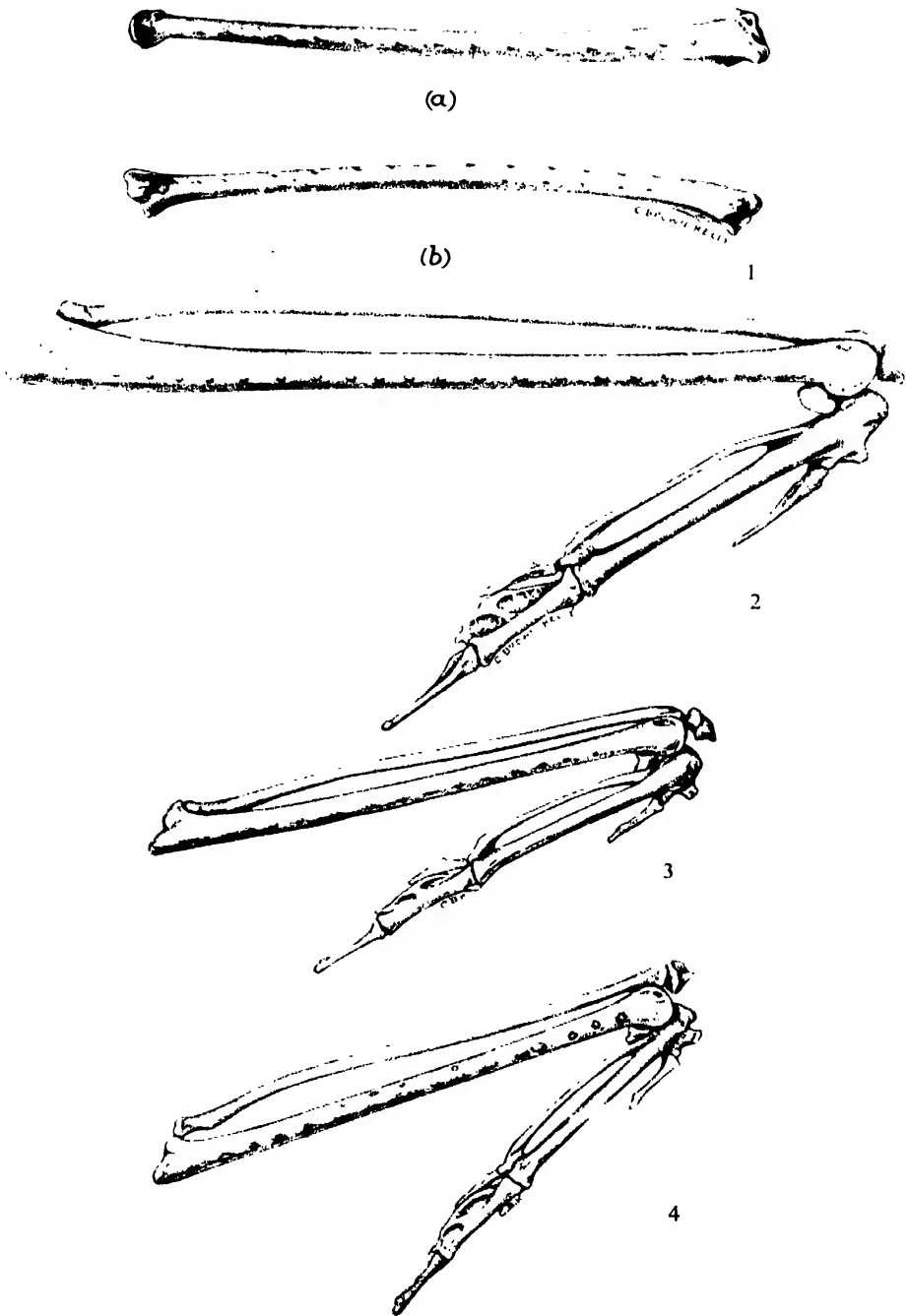
Fig. 20.—Similar section in nestling black-headed gull, *Larus ridibundus*. b., bony tissue of ulna, c.t., connective tissue attachment from periosteum (p.) to a feather rudiment (f 1); f 2 and f 3, other feather rudiments; ost., osteoblasts under the periosteum.

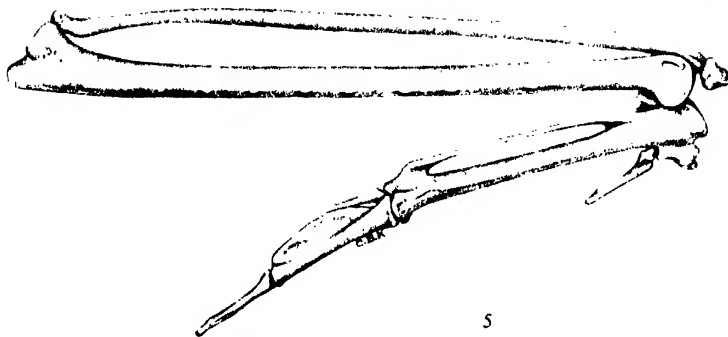
PLATE VIII.

Fig. 21.—Section in slightly older gull, running about with assistance from wings. f., feather rudiment; *, area with cells suggesting early cartilage; other indicators as in figs. 19 and 20.

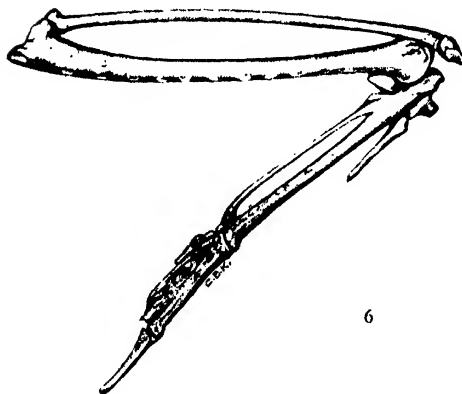
Fig. 22.—Higher magnification of area * of fig. 21.

(Issued separately September 16, 1941.)

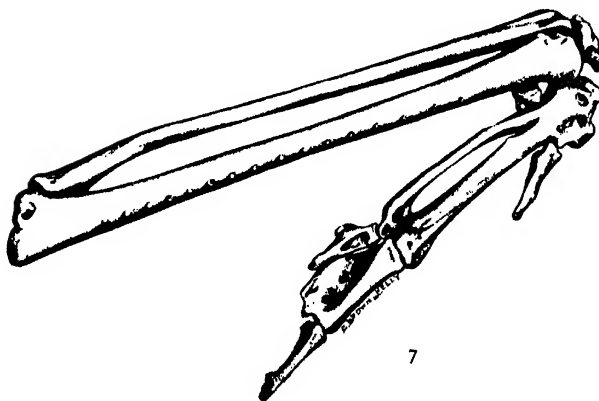




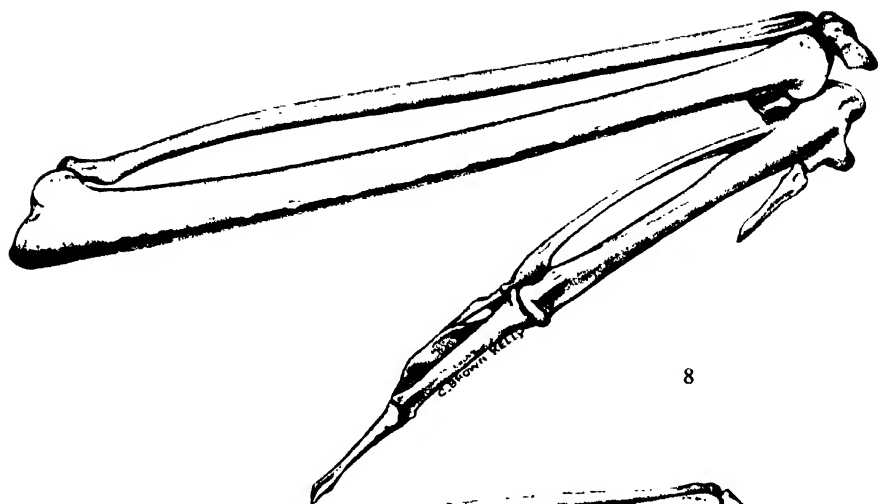
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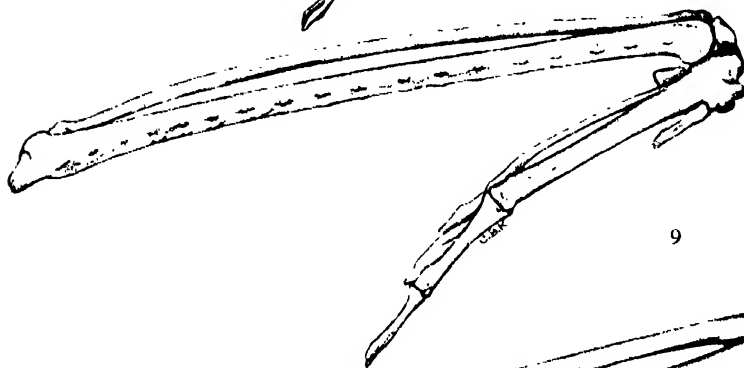
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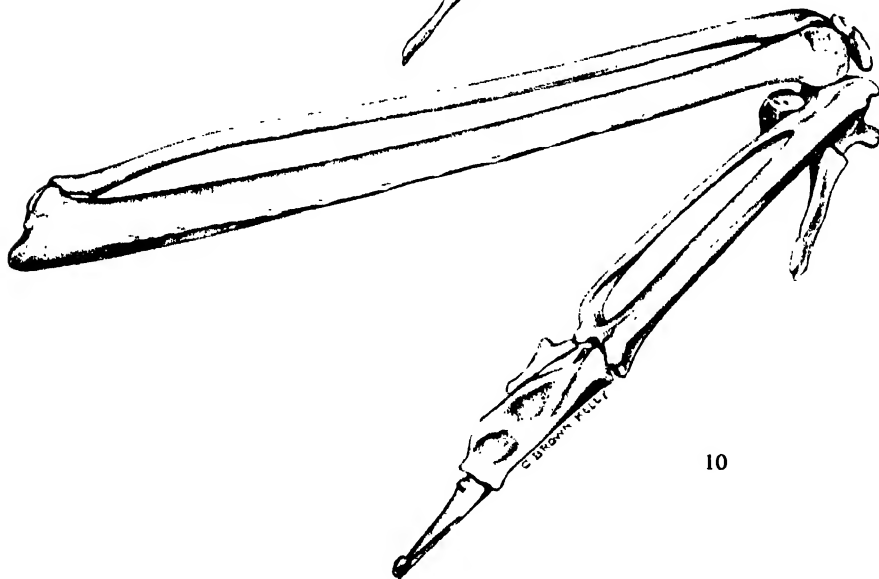
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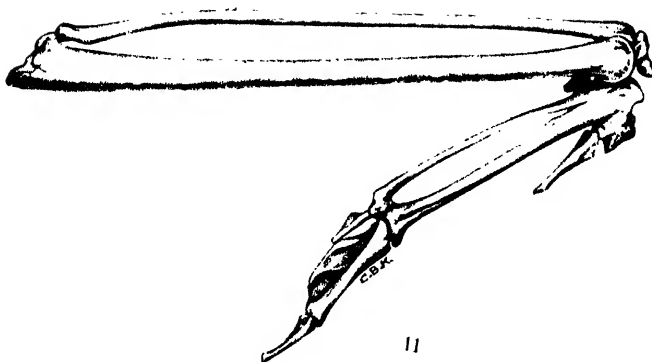
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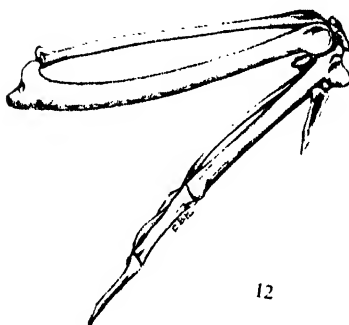
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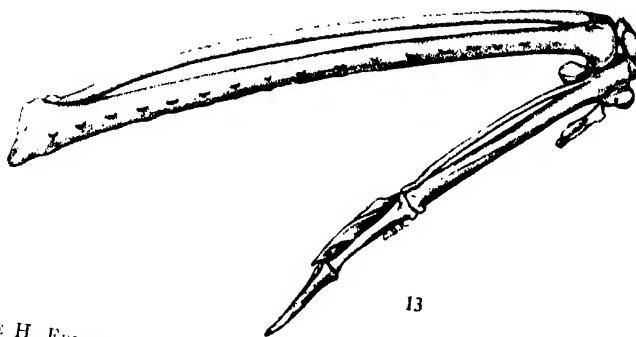
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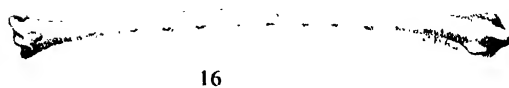
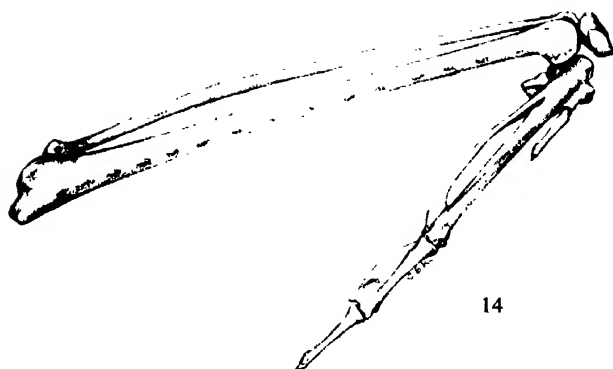
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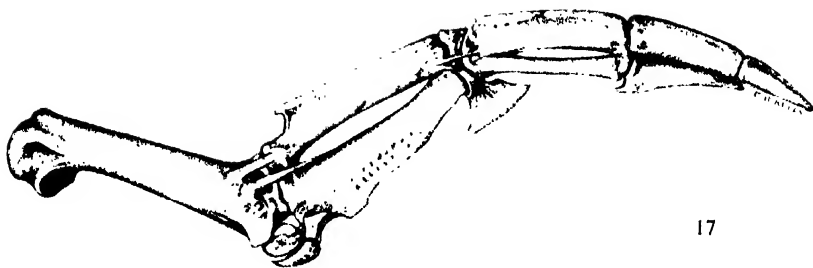


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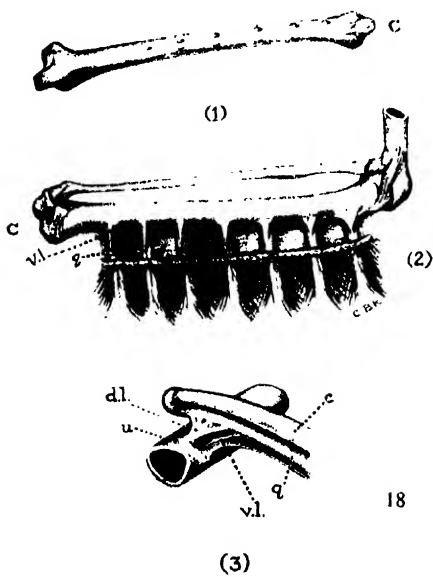


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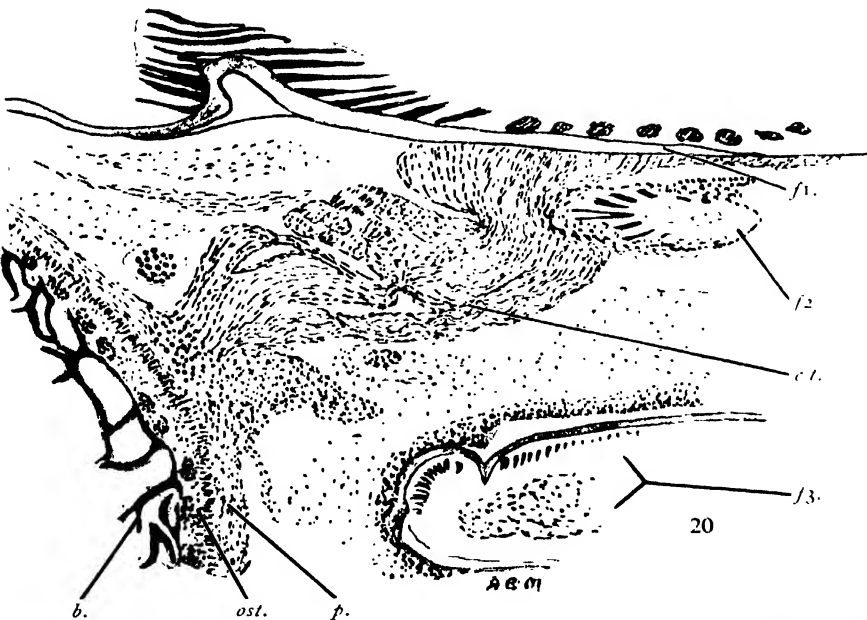
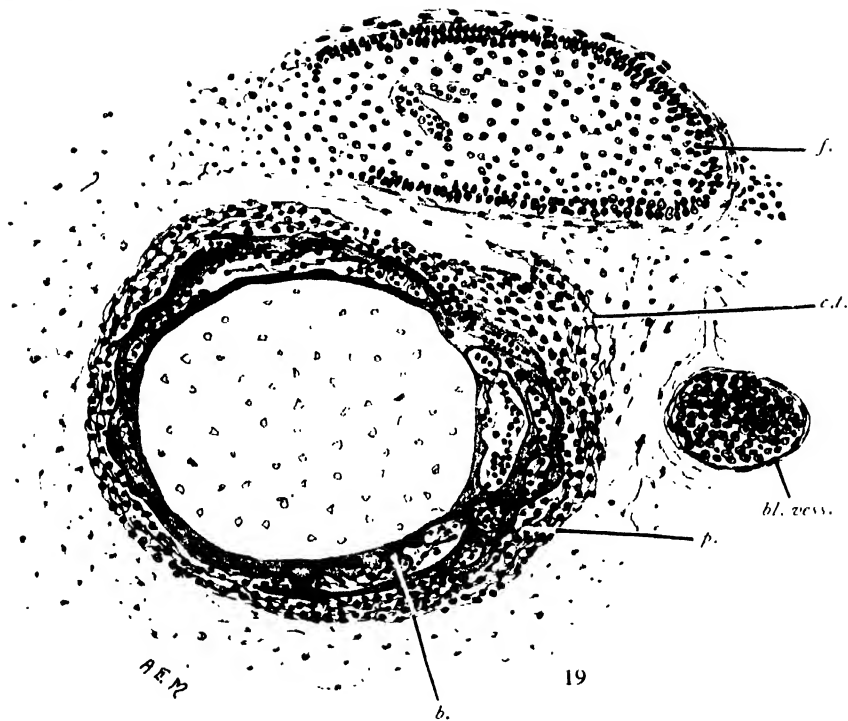


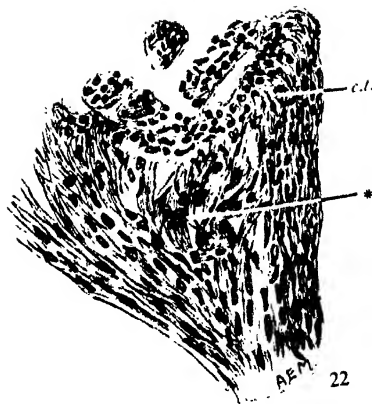
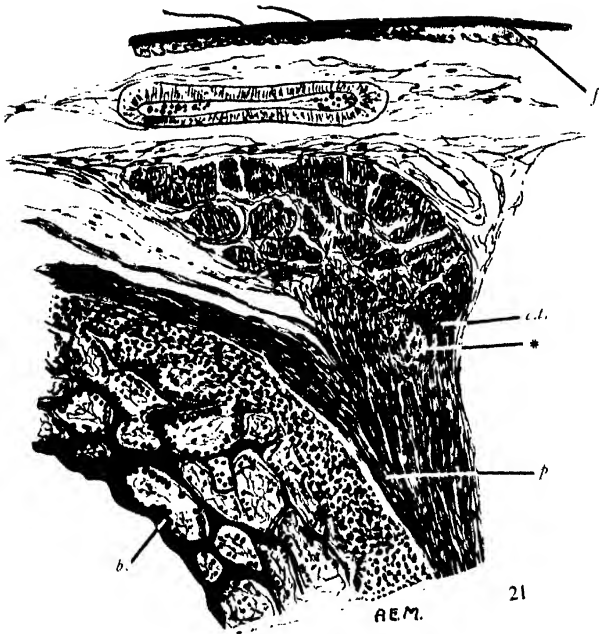


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XIII.—The Evolution of Continents: A Possible Reconciliation of Conflicting Evidence. The BRUCE-PRELLER Lecture for 1941. By **Sir Thomas H. Holland**, K.C.S.I., K.C.I.E., F.R.S.

(MS. received July 7, 1941.)

I. THE DOCTRINE OF PERMANENCE.

IN a letter to *Nature* published in 1881, J. D. Dana recapitulated his reasons for suggesting, first in 1846 and on frequent occasions afterwards, that "the continents have always been continents . . . and have never changed places with the oceans." This view of the permanency of continents and oceans became generally adopted by Sir Charles Lyell (1868) and other leading naturalists during the middle of the last century: it became recognised as what might be called the orthodox doctrine.

The only member of the "heroic" group of naturalists who expressed unorthodox views during the Victorian age was Edward Forbes (1846), who found it impossible to reconcile the distribution of animals and plants without great changes in the past between ocean and continent.

Charles Darwin (1886), however, specifically rejected the views of Forbes, when he had to reconcile what was known then of biological distribution with his own doctrine "that each species has proceeded from a single birthplace." He indeed realised that Dana's idea was a formidable snag to his theory, but hoped that further experiment and observation might explain how the animals and plants got across the oceans to plant their colonies on distant lands.

Alfred Russel Wallace, when he wrote Chapter VI of his well-known *Island Life* in 1880, accepted the doctrine in almost an extreme form, and Huxley (1870) too, in his Presidential Address to the Geological Society of London, would not demand for the migration of his vertebrates more than a marginal change in the level of the land to allow them the chance of spreading during Miocene times from Abyssinia to the eastern part of his so-called faunal province of "Hindustan."

In the opening paragraph of a memoir published nine years ago, Professor Charles Schuchert of Yale stated his belief "that both continents and oceanic basins are, in the main, permanent features of the Earth's surface; but that they have not always had their present shape and

area" (Schuchert, 1932, pp. 876 and 880). On a later page he explained in fuller detail that he did not accept the principle of permanency in the rigid form propounded by the elder Dana, for he assumed that land bridges must have extended across some oceanic basins to account for the distribution of past life.

II. THE FOUNDERING OF GONDWANALAND.

This then represents a long-lived and widely prevalent view, adopted as an alternative to the conclusion of W. T. Blanford, who made the first formal attack on the old doctrine of permanence, in his Presidential Address to the Geological Society of London in 1890. With a far larger stock of data than was available to Darwin thirty years before, he then pointed out that the known distribution of past life indicated something far more serious than merely marginal trespasses of the ocean on the land areas; that indeed wide stretches of ocean, with their present beds submerged a thousand fathoms and more, must have been wide stretches of continental land in past ages, either continuous or intermittently so. No naturalist has ever challenged Blanford's array of facts, but many, perhaps most, like Schuchert, have satisfied themselves instead with the existence of past land-bridge causeways for biological migrations between the established continents.

Blanford derived his idea mainly from the southern regions, where the oceans are wide and deep, with apparently no stepping-stones across them, such as we have in the North Atlantic between Europe and North America, and, by way of the Aleutian Islands, across the North Pacific to Asia. Australia, most of Africa and South America, Madagascar, the Falklands, and Antarctica all include records exactly corresponding to those of the Gondwana system of rocks of India, and in ways that point to old land connections of some sort between all of them. If these were all included in one area (Gondwanaland, as Suess named the supposed old continent) they would have had a total area as large as the whole of the present lands of the globe; and to raise the present southern ocean-beds sufficiently to restore Gondwanaland would displace enough water to cover all the remaining continents.

III. THE THEORY OF CONTINENTAL DRIFT.

One can understand therefore why some naturalists, who still hang on to the old doctrine of permanency, favour the device of land-bridge connections to satisfy Blanford's conclusions. In his time no one had seriously thought of the possibility that the continents themselves might

have drifted apart, and this idea Schuchert refuses, as most geologists still hesitate, to accept.

This new theory took a definite form most noticeably after the publication of the late Alfred Wegener's book (1915), but something of the sort was suggested as long ago as 1889 by Osmond Fisher. In a frankly speculative final chapter of his *Physics of the Earth's Crust*, he pointed out that if the moon had really left the Earth, as Sir George Darwin had suggested, the scar left behind as the Pacific Ocean basin would have resulted in a break-up of the continents, with a horizontal sliding of the adjoining lands to fill the newly formed hollow, and the consequent opening out of a new basin to form the Atlantic Ocean.

Evidently the facts of life distribution, whether past or present, would fit equally both parties—those who think that migration was facilitated by former land connections across the ocean, as well as those who cherish the new idea that the continental blocks of granitoid rocks have been floating about like rafts on a heavy, world-wide "ocean" of basic molten magma.

Like all new ideas which clash with long-established popular doctrines, this suggestion of continental drift has given rise to warm controversy, but mainly because Wegener and some enthusiastic supporters of his hypothesis went incautiously beyond their "last" and provided physical causes for the drift which geophysicists regard as quite untenable. The distinguished South African geologist, A. L. du Toit, published three years ago a comprehensive work entitled *Our Wandering Continents*, in which he is frankly aggressive to the so-called orthodox naturalists, assuming openly that the most effective form of defence is attack (du Toit, p. 2). As a consequence of this attitude, both critics and supporters naturally tend to dispute or to hold in abeyance many of du Toit's arguments which cannot be independently verified, or would be regarded as justifiable accessory evidence if the drift theory were definitely established as a "fact" in geological history.

Some of these forms of evidence which have been quoted by du Toit and are at present of doubtful value, should be mentioned briefly. For example, the supposed changes in the longitude of Greenland and Jan Mayen Island during the last sixty or seventy years do not seem to be pertinent to the question whether drift movements on a wide scale occurred late in the Mesozoic era and through Tertiary times, except to show that the continents are not even now rigidly fixed. Perhaps they are not; but geodesists justifiably regard these recorded determinations as possibly due, not to actual movement, but merely to greater precision being adopted in the latest methods of observation. And the significant circumstance

that the later observations all agree in showing a westerly movement is again partly discounted by the fact, which du Toit himself has rightly mentioned (1937, p. 300), that there are no recorded changes in latitude. The same caution applies to the longitude values recorded for Sydney between 1883 and 1926. To use this form of evidence it may be necessary to wait for many years, and then to rely only on precise observations made, as suggested by Dr L. Hawkes (1935), on the stable continental shields.

Similarly, evidence from the distribution of animals and plants is full of pitfalls and must accordingly be treated with caution, because we have very little information about the ancestry, and therefore origin, of the peculiar land forms which are now mainly limited to the separated continents; for example, the present limitation of monotremes, marsupials, and struthious birds, like the emu, ostrich, and rhea, to the southern continents. These, like the distribution of rain-worms, scorpions, and other land forms, would have a simple explanation if we could otherwise and independently prove that the continental masses on which they now live were once in close proximity to one another.

Controversy about the drift theory has thus too often obscured the facts by criticism of suggested causes and even by criticism of the controversial methods of partisans. But only rarely does the world produce a Charles Darwin who is content to pile up facts for twenty years before publishing a new theory.

IIIA. *The Glaciation of Gondwanaland.*

There are, however, even at this stage, some lines of evidence which cannot, so far as anyone knows, be explained in any way except by continental drift, and the most formidable among these is the record of contemporaneous glaciation on all the southern continents as well as in India. Blanford, who first recognised the record of ice-action in the Palæozoic rocks of tropical India in 1857, and was scoffed at accordingly for nineteen years, had no satisfactory explanation for the fact that glacial conditions—often certainly at or near sea-level—occurred in Upper Carboniferous times on all preserved parts of the supposed old Gondwanaland, as far south as the Cape in South Africa and as far north as the Punjab in India; that is, both south and north of the Equator, as well as at several places within the present tropics. For one of these glacial horizons, namely, that corresponding to the base of the Gondwana system in India, the evidence for chronological contemporaneity is as simple and as precise as anything we can hope for in stratigraphy (Holland, 1933); it is now known unmistakably in four of the Australian States, in South

and Central Africa, Madagascar, the Falkland Islands, in the Argentine, Uruguay, and tropical Brazil, as well as in India.

Before the suggestion of drift was made, this Palæozoic glaciation was a baffling mystery, and all sorts of explanations were tried, each in turn breaking down when tested by the facts. Not one among them but the assumed drifting of the continents has so far survived criticism. Various attempts have been made to juggle with changes in latitude; but no scheme could get over the simple fact that the Indian "tillites" are now scattered through tropical India and up to as far as 30° north of the Equator, whilst others on the southern continents are as much and even more to the south. On the other hand, if the present continental masses had drifted away at some geological age later than Palæozoic times from near the Antarctic region, and carried with them their old glacial boulder beds, they quite naturally would have taken with them also the remains of the contemporaneous animals and plants.

In addition to the glaciation referred to as Upper Palæozoic, other much older ones are recorded; for instance, in the Devonian as well as in the pre-Cambrian rocks of Australia and South Africa. These records are now found lying between 65° to 70° away from the present South Pole; and, as it is unlikely that the Antarctic ice-cap ever reached so far as the tropical zone, such occurrences can fairly be added to others as consistent with the drift theory.

All-world refrigerations doubtless occurred at various times in the distant past, as it did in the so-called Great Ice Age of the Pleistocene period; but, as Sir George Simpson (1930) has urged with recognised authority, the climates would always have been zonal from a relatively warm equator to the colder polar regions. Thus, if glacial conditions prevailed at sea-level in the tropics, the world would have been encased in ice, which we know to be untrue for all past time. And it is significant that similar glaciations occurred also during these very old periods (pre-Cambrian and Older Palæozoic) in the corresponding northern regions, especially in Canada and Scandinavia, although we cannot precisely correlate the southern with the northern instances in the time-table. But during Upper Palæozoic times (that is, corresponding to Lower Gondwana times in the south) there were no undoubted records of glaciation at sea-level in the northern regions, the inference being that most of Gondwanaland was then still within or near the Antarctic circle. On the other hand, there are records of laterite of Upper Palæozoic age at intermediate low latitudes showing the existence there of tropical conditions when Gondwanaland was mostly under ice.

A theory of continental drift would be simple and convincing if we

could only find the motive force: how and why it happened. Still, it may be true even if we cannot explain why; for there are many other forms of evidence which suggest independently that the continental masses, now widely scattered, were at one time much nearer one another, perhaps parts of a relatively small continent in the south: that, indeed, there was a Gondwanaland after all!

III B. *Pre-Cambrian Correspondences.*

The very old pre-Cambrian rocks in all parts of Gondwanaland show, for example, several points of striking resemblance, which alone of course would be of small significance; for sandstones, shales, limestones, and metamorphic rocks are not exclusive or always distinctive; but there are nevertheless many features in common which are peculiar to the southern continents, such as the Rand gold-bearing banket and the Nullagine series of Australia of about the same age.

III C. *Siluro-Devonian Sandstones.*

Somewhat stronger, but still not in itself conclusive, evidence of another sort is offered by instances such as a persistent and thick sandstone formation ranging from Upper Silurian to early Devonian times in New Zealand, South America, and South Africa, where a part of it is well known as the Table Mountain Sandstone. This formation is peculiar, conspicuous, and impressive, just as the Old Red Sandstone is to us in parts of Great Britain, Russia, the Baltic areas, and North America; and its existence on different continents naturally suggests that Gondwanaland once occupied a much smaller area than one would suppose from the distances by which its fragments are now separated.

III D. *Gondwana Correlations.*

When one searches for records further up in the time scale, above the base of the Indian Gondwana system (that is, the Upper Carboniferous horizon of a wider terminology), one finds still more striking correspondences, especially in the order of stratigraphical succession, such as the coal measures in the Lower Gondwanas, with several identical species of *Glossopteris* and *Gangamopteris*, on all the continents, including Antarctica; and above the coal measures there are similar red beds which indicate the prevalence, in late Permian and Triassic times, of arid and semi-arid conditions simultaneously in India, Madagascar, South Africa, and South America.

With this group of evidences, one very striking feature in common is the great spread of plateau basalts—what G. W. Tyrrell calls “flood basalts”—which were erupted near the end of the Triassic period and are preserved in the Stormberg series in South Africa, the Serra Geral series in Brazil and Uruguay, the Rajmahals in India, and the dolerite sills in Adelie land, all of about the same age and on an enormous scale.

A map which du Toit published in 1927, following a special visit to South America, compares the opposite shores of the South Atlantic from Patagonia to the Guianas on one side, with Africa from the Cape to Sierra Leone on the other, showing how closely the formations correspond in their peculiarities, stage by stage, up to the marginal marine transgression at the end of the Mesozoic era, and showing too a significantly remarkable agreement in the order of succession, as well as the detailed matching of the subdivisions, lithologically and palæontologically. This extraordinary correspondence between the two areas impresses everyone who has seen parts of both areas, although no single person has gone over all the ground on both sides of the Atlantic.

Then again, the parallel tectonic lines on both sides of the ocean suggest fold movements which probably affected a single continental mass originally; for they could hardly have occurred independently on lands so widely separated from one another as they are now.

A pretty instance, chosen from many, will serve to illustrate another kind of comfort to be derived by supporters of the drift hypothesis, as well as the inexplicable inconveniences in theory which would arise if we were bound to assume that the continents were always as widely separated as they are now. In Cape Colony there is a thin band of black clay in the Dwyka series; and at the same horizon in South America the Iraté shale is composed of similar material, also forming a thin bed, both being limited in distribution. In each place there are well-preserved remains of a small primitive, free-swimming reptile, *Mesosaurus*—a delta scavenger—which is not known elsewhere in the world. If South Africa and South America were always separated as they now are by an ocean 4000 miles wide, it is very unlikely that the bones of *Mesosaurus* would have been found in both areas, and in similar deltaic clays very nearly, if not exactly, of the same age. If, however, these two continents were at one time close to one another, *Mesosaurus* would naturally have found it possible to get around from one river delta to another near by. What we can say justifiably at present with regard to the evidence of *Mesosaurus* is that his occurrence can reasonably be regarded to support the idea that South America and South Africa were at one time much nearer one another than they are now, near enough to permit of the migration of an animal that

certainly could not travel overland and almost certainly would not have attempted an ocean journey.

III.E. *Contrast of Glossopteris and Gigantopteris Floras.*

In recent years there has been a considerable increase of our knowledge of the fossil plants preserved in the Permo-Carboniferous and later formations in Western China, Indo-China, and Sumatra, and these new data again quite unexpectedly form another important piece of circumstantial evidence which fits in with the idea that this part of the Asiatic region, in later Palæozoic times at least, was separated from Gondwanaland by a barrier which could not be crossed by migrating plants.

The assemblage of Palæozoic fossil plants of this Indo-China region is generally known as the *Gigantopteris* flora because of the prevalence among the fossil plants of that remarkable genus, and quite recent critical examinations by T. G. Halle, B. Sahni and others show that, contrary to previous impressions, the *Gigantopteris* flora contains no species in common with the widespread *Glossopteris* flora of Gondwanaland; that the plants which lived in the region where *Gigantopteris* flourished indicate the existence then of a warm, moist climate with luxuriant vegetation, whilst the *Glossopteris* flora lived in a comparatively cold climate. According to Sahni (1938), "we know of no other two floras, living or extinct, which are floristically and climatically so distinct; and yet they now lie side by side on the map; crossing the same latitudes along a north-south front of well-nigh two thousand miles."

This evidence from the fossil plants, found unexpectedly only two or three years ago, is in striking agreement with the assumption that the part of Gondwanaland which is now contiguous with the Indo-China region was widely separated from it in late Palæozoic times. And no barrier that we know of could have prevented the intermingling of plant species with such different climatic habitats so completely as a wide stretch of ocean, which must have existed between the two regions in Palæozoic times if continental drift did actually occur in the way postulated by supporters of that hypothesis.

These selected samples of evidence seem to require, so far as our present knowledge goes, continental drift on a large scale for their explanation. They also seem to be quite independent of one another; the failure of any one of them to stand cross-examination would not apparently invalidate any other. There are, of course, many other witnesses that might be called, and there is a still larger number of observations which could be explained satisfactorily if it were legitimate to admit that drift is physically possible on an Earth constructed as it seems to be to-day. Even, however,

if no single piece of evidence now quoted could be regarded as crucial, when considered alone, the "cloud of witnesses" telling a similar story is surely too significant to be put aside lightly.

IV. GEOPHYSICAL OBJECTIONS TO THE THEORY OF DRIFT.

Yet, H. H. Jeffreys objects, from evidence mainly revealed by seismic waves, that the physical state of the subcrust would render large-scale horizontal movements of the sort contemplated by the theory of continental drift to be quite impossible, that "such motion is opposed by the strength of the matter near the surface which is open to no doubt whatever" (Jeffreys, 1935, p. 173).

And this simple statement has just as much right to be classed among "facts" as those which have been quoted in support of the theory; * for it is founded on a mass of consistent data which show that the Earth is solid and strong down to about half its radius.

A paper on Seismological Tables recently published by Jeffreys (1939) may be taken as an illustration of the way in which the geophysicists are gradually narrowing their earlier points of difference in detail and have attained increased precision in their interpretations of seismograms since R. D. Oldham (1900) first separated the longitudinal, transverse, and surface waves emerging from a typical earthquake. This paper is mentioned here only to show that the degree of strength as well as rigidity can be relied on for the production of trustworthy results from the evidence of earthquake waves. In other words, the physical state of the Earth which Jeffreys has in mind is an observational fact to be admitted as scientific evidence.

V. POSSIBLE RECONCILIATION OF CONFLICTING EVIDENCE.

On the other hand, the geological records which have been quoted were chosen because they also seem to be facts which, so far as one can judge, admit of one simple and single interpretation, namely, that the present continents must have been moved over great horizontal distances. Thus, we have two groups of natural "facts" which are apparently inconsistent with one another; so there must be a "catch" somewhere; and this we should, if possible, try to discover.

There is one obvious direction in which we might look hopefully for

* A "fact" in science is often only an occurrence reasonably deduced from circumstantial evidence. "Every statement of fact involves certain general notions and theories, so that the 'facts' of the separate sciences cannot be stated except in terms of the conceptions or hypotheses which are assumed by the particular science" (A. S. Pringle-Pattison).

a clue, and that is this: the evidences for continental drift all belong to a relatively distant past; they began not earlier than about 100 million years ago towards the end of the Mesozoic era and continued through much of the Tertiary period. The seismic records, on the other hand, indicate the state of the Earth's interior to-day. Yet it is true, beyond question, that such recurrent phenomena as the rise of great mountain chains, the outflow of enormous floods of basalt, and the occasional transgression of the ocean over wide stretches of the continents have actually occurred repeatedly in the past, and on scales wide enough to necessitate a review, and possibly revision, of the ideas which are based on the present stage in the evolution of geomorphology.

It seems necessary then at this stage to consider briefly whether these older phenomena were on a scale sufficient to suggest that some at least of the geophysical evidence to-day may be under suspicion when used as a test of the past. There seems to be no evidence that anything akin to the alleged Mesozoic-Tertiary drift occurred at any earlier period in the Earth's history; indeed, the observations on which the theory is based show that certain continents, which are now widely separated, were near one another back at least for some 800 million years—that is, nearly as long before the appearance of fossils in the Cambrian period as since.

VI. THE CONCENTRIC EARTH LAYERS.

We might first review briefly at this stage the kind of evidence which has to be taken into account in forming an idea of the physical state of the Earth below what might be called the geological "sphere of influence." All agree generally in recognising two main layers in the outer crust of the Earth—an outermost, more siliceous, and incomplete layer, forming the continental masses, lying on a lower, more basic, complete envelope. Following the terminology suggested by Eduard Suess, these are often referred to by the dominant bases in their silicates as *sial* and *sima*. The geophysicist gets his information from below the visible rocks, mainly through the seismoscope, which is to him what the spectroscope is to the astronomer; one analyses earthquake waves to estimate the physical, and, within limits by inference, the chemical, nature of the Earth's interior; the other uses light-waves to study the nature of the stars.*

The outermost and most siliceous part of the crust is often referred to as granite, but in average composition it more nearly approaches the rocks grouped roughly as granodiorites, that is, rocks with about 65 per

* This pretty analogy is due to R. D. Oldham (*Quart. Journ. Geol. Soc.*, vol. lxii, 1906, p. 456), but has been at times put to the credit of H. Benndorf (*Mitth. Geol. Gesellsch. Wien*, i, 336, 1908), who used it two years later.

cent. of silica instead of over 70 per cent. It is what the stone-mason rather than the more narrow-minded petrologist would call granite.

Dr E. M. Anderson (1938) refers to the Lewisian gneiss of North-West Scotland as a fair type of the average sial in composition, but I would suggest that a much more homogeneous example on a far larger scale is afforded by the prevalent member of the charnockite series, which has a mean silica percentage of 64 instead of 70, and a density of 2.77 instead of 2.67. Over forty years ago the charnockite series were found to form the chief mountain ranges of South India and Ceylon; and similar rocks have since been found on a large scale in Central Africa as well as in Antarctica. They are remarkably uniform in their general characters, and are so suggestively peculiar among crystalline rocks that their inclusion among normal igneous rocks has sometimes been questioned (Groves, 1934). Their appearance only in the stable "shields" which have been exposed by uninterrupted erosion for long geological ages has tempted many to suggest that they are samples of a relatively low and anciently formed stage in the primitive sial shell. Such speculations, however, may well be held in check until the actual age of the charnockite series can be determined by their radioactive products.

The existence of a basic magma layer below the sial, forming a complete Earth shell, is a quite old idea among geologists. Even as long ago as 1844, Charles Darwin (1891), whose imagination travelled so far ahead of his times, made the suggestion that a basaltic or basaltoid substratum underlies the granitoid outer crust. The subdivision of this shell into two layers of ordinary and olivine-bearing material was described in 1938 by Dr W. Q. Kennedy and E. M. Anderson in the two complementary papers already quoted, which are likely to have historical value in geophysical science, as they will also prove to be of fundamental importance in putting the classification of igneous rocks on sound scientific lines based on their genetic descent.

And this is important to us in this discussion, because one division of igneous rocks is derived directly from the outwelling of the basic magma in the form of plateau or flood basalts, whilst the other division is derived mainly from the granodioritic sial layer and is formed during mountain-building processes.

Both sources of igneous rocks provide products by segregation which in mineral composition may resemble one another: basic rocks may segregate from the granodiorite magma, whilst siliceous, as well as ultra-basic products, may form locally from the basaltic magma.

Anderson, in his contribution to the joint paper, gives reasons for assuming that temperatures not far below those of fusion exist at a certain

depth underneath the continental crust of sial, requiring only slight augmentation by energy of radioactive origin to produce actual melting; that the outer layer may be 10 to 13 kilometres thick under the continents; and that the basic underlying material may persist downwards for about another 25 kilometres.

The seismic evidence supports to some extent the subdivision of this basic intermediate layer, but the earthquake waves indicate a much more definite physical discontinuity at its base, where it rests on material of presumably ultra-basic composition. There are various views about the precise mineralogical nature of this lower, presumably ultra-basic, layer which persists down to nearly 3000 kilometres from the surface, where another major discontinuity is indicated by seismic waves between it and the core of the Earth, which is generally considered to be composed mainly of nickel-iron still in a molten state, for, as Oldham first noticed, it is unable to transmit distortional seismic waves.

VII. HEAT OF RADIOACTIVE ORIGIN.

With the discovery of radioactivity there came first a reconciliation between the geologists and physicists regarding previously irreconcilable estimates of the time interval between the stage when the Earth was a molten globe and the time when it was possible for life to flourish on its surface. But that radioactivity meant more to the geomorphologist than a lengthened time-table was quickly appreciated. Lord Rayleigh showed, in 1906, that the radioactive elements were mainly concentrated in the outer shells of the Earth, and Joly (1923) followed with the suggestion that in every 40 or 50 million years there must have been an accumulation of sufficient heat in the subcrust to cause world-wide fusion of the basaltoid layer to form a mobile "ocean," followed by movements due to tidal stress, the outwelling of the basic magmas, and ultimate refrigeration to prepare for the next similar revolution in physical geography.

The recurrent, but not regularly periodic, wide trespasses of the ocean on the continental lands, followed by recessions consequent on Joly's line of reasoning, are recorded clearly in geological history; but probably no one now accepts his theory in its entire form—that is to say, a world-wide melting of the basaltic layer at regularly recurring intervals.

VIIA. *Flood Basalts.*

Nevertheless, on various occasions in the past this intermediate layer was melted over areas almost certainly far greater than that covered by the lava which flooded over the surface, for the flows of any appreciable

age have been largely destroyed by subsequent weathering, or, like those of North-West Europe, have since been covered by the sea. In the Paraná basin of South America the lava sheets still preserved cover over 300,000 square miles; in Oregon and the adjoining Western United States and on the Indian Peninsula outflows of the same order occurred, the flows mounting generally to a mile or more in total thickness. Further back in geological history, outflows of the sort and size have occurred in several periods both before and since Cambrian times. In addition to the lava flows which have emerged at the surface, enormous quantities have remained filling in the swarms of dyke fissures, which are found well beyond the area of what is still left of the visible surface flows, indicating that the intermediate layer of basic rock magma below must have become molten over at least equally wide areas.

VII B. *Marine Transgressions.*

Joly pointed out that, on melting, the basaltic magma would increase by 10–12 per cent. in volume and that the actual fusion would be relatively sudden. That being so, melting over any considerable area would result in extensive fissuring of the granitoid layer above, with the injection of swarms of dykes, some warping of the surface, and the production of simple monoclinical folds. With this change in volume and therefore density, the continental blocks would sink into the basic intermediate layer and there would follow a transgression of the ocean over the surface of the land. Transgressions of the sort certainly did occur in geological history, as, for example, that which affected the North American continent in Laramide times, and that which preceded the outflow of the Deccan trap on the Indian peninsula. The occurrence of these marine trespasses on an extensive scale gives support to the idea that melting took place over very wide areas before the eruption of flood basalts; but it cannot be said that they were world-wide, as Joly seemed to think, and fortunately for the world they were not so.

VIIC. *Folded Mountain Ranges.*

In another way the rise of mountain ranges, such as the Alps, Himalayas, and American Cordilleras—all within quite late geological times—shows that the physical state of large parts of the Earth in Tertiary times was widely different from its condition to-day. Similar orogenic phenomena on a large scale appeared in older periods, whilst their recurrence since shows no tendency for the intermediate periods of rest to lengthen as would be expected if the potential energy in the outer shells had been dissipated throughout the ages to any appreciable extent.

The strata which now form the great mountain ranges were laid down in trough-like depressions hundreds of miles long, which continued to subside intermittently many thousands of feet, and through some millions of years, whilst the alluvial deposits accumulated in them. But no one can say why such geosynclines were started, why they continued to develop for ages, why the downward movement finally stopped, and why thereafter the continental masses on either flank of the depressions closed in with irresistible force horizontally over scores of miles, to crumple up the beds, and—still more mysteriously—why, after the crumpling was accomplished, the bundles of strata were lifted up to form mountain ranges as high as the Himalayas. They are historical facts all the same, and are as difficult to explain as the supposed drift of the continents over thousands of miles.

It is important to keep in mind the fact that these large-scale troubles, which have recurred at intervals in the past, seem to have had their origin in the two outer layers of the Earth; and it is insufficient to say that they were merely due to the crust collapsing on a shrinking cooling core. That does not explain the long intervals which elapsed without such gigantic movements; and even if the crushing of the great geosynclines by movements mainly at right angles to their general alignment were the outcome of shrinking, there is still no explanation of the subsequent rise of the mountain ranges which all seem to have granitic cores. Heat from the degeneration of radioactive elements, accumulating through ages, finding escape by geotectonic revolutions, was the central idea of Joly's theory, and in some form his speculations in part are likely to offer a solution.

VIII. STRENGTH OF THE SUBCRUST.

Yet, when we attempt to correlate these ideas with the theory of continental drift, we are faced with abundant evidence to show that the outer shells, as well as the rest of the Earth down possibly to half its radius, have now strengths comparable with that of the surface rocks. Seismic records of normal earthquakes originating at relatively shallow depths show, by the transmission of distortional waves, that the intermediate layer as well as the continental granitoid crust above it is quite solid. And since H. H. Turner detected them first in 1922, even deep-focus earthquakes have been recorded down to quite 700 kilometres. Although they are still few in numbers, they indicate that the strength of the Earth to this depth is also comparable with that at the surface, for the earthquakes must have originated by relatively sudden fractures, following the local accumulation of sufficient stress-differences. The waves sent out from such deep-focus earthquakes have also been characterised by a dominance of the distortional kind which could not have arisen from a molten magma.

VIII A. *Origin of Deep-focus Earthquakes.*

Jeffreys (1935) considers that thermal changes at great depths are quite sufficient to account for the stress-differences needed to produce these large deep-seated earthquakes, and this idea fits with his older conclusion, obtained on other grounds, that the cooling of the outer shells of the Earth accounts sufficiently for the folding of mountain ranges to accommodate the outer crust to the shrinking body. But contraction due to a falling temperature in the lower layers must necessarily be a slow process, and one would expect therefore that stress-differences so created would be adjusted before sudden fracture occurs under the great pressures that ought to prevail at depths of 700 kilometres.

A more plausible explanation, at least to the petrologist, seems to be offered by Fermor's suggestion (1913) of a paramorphic change from compounds of high to others with lower molecular volume, and this is a kind of change which might well occur with relative suddenness when local or even fairly widespread conditions reach a critical stage of temperature and pressure in a body which is composed dominantly of minerals liable to such reversible changes of "volume."

Fermor used as his chief illustration the mineral garnet and the possible formation under pressure of the rock eclogite (in which garnet predominates) from a basic magma, because eclogite is a rock type actually known to exist not only at the surface, but is found in the kimberlite of the diamond pipes, which has been brought up from great depths and contains other minerals of a very low molecular volume—the diamond for example.

Jeffreys (1935, pp. 132, 133) suggests, instead of eclogite, the existence of "a high pressure modification of olivine as a likely constitution for the matter between a depth of 300 kilometres and the core"; but, although rocks of this class are known to be more compressible than granites, we know of no solid compound of the general formula $(\text{Fe.Mg})_2\text{SiO}_4$ with a lower molecular volume than that of olivine, which seems in all surface specimens to be just the sum of the volumes of the three chief constituent oxides.

With a faith in the efficacy of magma differentiation which an experienced petrologist is not likely to share, Jeffreys also suggests that eclogite should be ruled out because "it is a mixed crystalline rock, and a magma with the general composition of eclogite would separate in crystallization into layers." This suggestion will no doubt disappear from later editions of a work which seems to be hurriedly packed with some other bright speculations. It seems to be a frequent habit to speculate

from the nature of seismic waves regarding the mineralogical character of the "lower layer," but the practice is justifiable only when regard is paid to the limiting fact that miscible silicates refuse to segregate their mineral constituents cleanly, except under circumstances more favourable than petrologists know of at the surface. More likely the recorded seismic wave velocities with deep-seated paths represent the averages of very composite rock types, although not necessarily as roughly mixed as those of the stony meteorites.

If the differentiation of magmas were as uniformly clean-cut as some geophysicists seem to suppose, and as the apparently abrupt discontinuities between the Earth shells suggest, chemists would probably never have discovered near the surface as many as 89 out of the 92 possible elements, including the platinum family of metals which associate normally with the olivine rocks. And we would almost certainly be forced also to provide somewhere for a sulphide layer, as V. M. Goldschmidt suggested in his lecture to the Royal Institution in 1929; for the sulphides are not miscible with the silicates, and their separation from a magma is thus not so complicated; yet, they appear both as isolated grains in all basic igneous rocks, and occasionally even as local magmatic concentrations, still mixed with basic silicates, to form workable ore-bodies, such as the nickel-bearing segregations of Sudbury in Ontario. Quite obviously the sulphides which ought to be nearly as deep as the central metallic core occur in considerable quantities as far out as the basic intermediate layer.

Favourable conditions for segregation also account for the concentration of radioactive minerals in the outermost layer; but unless these conditions were perfect, there may well be still enough radioactive material at depths sufficient to justify a theory of convective currents, like that proposed by Holmes (1928-29) to account for continental drift as one way in which the accumulated heat has been intermittently dissipated.

IX. CONCLUSION.

Obviously, it was easier to speculate when our data were more limited and wanting in precision. Between 1743, when Clairault published his celebrated theorem, and 1900, when R. D. Oldham detected the three types of waves in actual records of earthquakes, mathematical physicists provided us with fancy Earths made up of every possible permutation and combination of solid, liquid, and gas. Since Oldham's recognition, however, of a central core which failed to transmit distortional vibrations, progress in the acquisition of definite facts by seismology has been positive;

and, at the same time, with more information about the distribution of radioactive minerals, progress has been rapid.

Nevertheless, the seismic evidence is still very limited and mainly obtained from records on continental areas. Very little is known either geologically or geophysically about the state of affairs under the Pacific, which covers nearly half the surface of the globe. Every science worker knows that it seems often easier to formulate an apparently satisfactory generalisation when the data are few than when they are abundant; and for our present thesis the moral is double-edged: more observations free of preconceived prejudice are wanted to check those which point significantly to continental drift, as well as more seismic records to control those which have so far been obtained. We still have very little information about the electro-magnetic condition of the Earth, and, until it is better understood, it may be unwise to assume that the geological evidence for continental drift is unreliable.

However, so long as geophysicists give good reasons for denying the physical possibility of continental drift, there are only two courses open to observational naturalists: (1) to suggest a reconsideration of the possibility, which is suggested by geological history, that some of the recurrent revolutions in the past were on a scale large enough to put the evidence of present-day conditions out of court; and (2) to continue with the prosaic task of accumulating more cold facts until, if ever, we can be more certain than, say, Laplace was in 1796, when he spoke of the Nebular Hypothesis as "conjectures presented with all the distrust which everything which is not a result of observation or of calculation ought to inspire."

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XIV.—Quantitative Characters of the Growth and Development of a Paurometabolous Insect, *Dixippus (Carausius) morosus* Br. et Redt. I. The Loss of Water in relation to Ecdysis. By **Beverley N. Smallman, M.A., Ph.D.**, Department of Zoology, McGill University.* *Communicated by Professor J. RITCHIE, M.A., D.Sc.* (With Four Text-figures.)

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INTRODUCTION.

WATER economy is a necessary complement of terrestrial life, for the limits of life upon land are, in part, determined by the organism's ability to utilize and conserve the water of its environment. For small organisms of terrestrial habit, the large surface-to-mass ratio and consequent surface evaporation makes the problem of water conservation particularly acute. It is therefore remarkable that the insects constitute the most numerous and varied group of terrestrial animals; and it follows that their success is, in large part, due to their ability to utilize and conserve water in widely divergent environments.

Insects may lose water from the body surface and the alimentary canal. Adaptations for water conservation may therefore be sought at these places. Thus, the integument of most insects is waterproof (Wigglesworth, 1934) and water-loss from the body surface (in dry air) is related to respiration and the resultant opening and closing of the spiracle sphincters. The control exercised here is mechanical, and any factor which tends to reduce respiration, such as prolonged starvation (Buxton, 1930), will automatically reduce the water-loss from the body surface and permit the insect to survive for long periods. Water-loss from the alimentary canal is reduced in a large number of insects by reabsorption of water from the contents of the rectum and by the excretion of uric acid which is insoluble and can be eliminated without water (Wigglesworth, 1932). Thus the meal-worm, which is capable of surviving long periods of desiccation (Buxton, 1930), excretes uric acid and releases the faeces as a dry powder (Wigglesworth, 1932). Babcock (1912) has indicated that grain weevils, clothes moths, and other insects living on very dry foodstuffs may retain and use the water of metabolism.

* The work reported in this paper was carried out at the Department of Zoology, University of Edinburgh.

Buxton (1932) has summarized the literature on the water relations of terrestrial insects.

The time of moulting in insects constitutes a period in which the organism would appear to be particularly vulnerable to water-loss by evaporation from the body surface. If the moulting fluid were lost at ecdysis, and if the new cuticle remained permeable to water for some time, the insect would suffer severe loss of water. Wigglesworth (1936) alone, has considered the water-loss at this important period. He has shown that, for the imaginal moult of *Rhodnius prolixus*, the extra loss of water is small, due to the almost complete reabsorption of the moulting fluid and the near impermeability to water of the new cuticle before the old cuticle is shed.

At the cessation of feeding, prior to ecdysis, the insect loses its means of gaining water while evaporation and defæcation (until the gut is almost emptied) continue to eliminate it. Thus it seems that at this important stage of its development the animal must lose considerable amounts of water, to the detriment of its water balance. However, Teissier (1931) has shown that the act of moulting produces no change in the proportion of water in the meal-worm. The meal-worm may present a special case, but it seems probable that terrestrial insects, exhibiting a consistent water economy, will have developed some means of maintaining their water balance at the moult.

Loss of water from the alimentary canal of insects has not been studied quantitatively. Wigglesworth (1932) has shown that it is to some extent controlled. Moreover, large differences in the amount of water lost from the alimentary canal are found in different groups of insects (Wigglesworth, 1932) and it appears that changes may be induced in individuals (Wigglesworth, 1933 a).

The present study presents data regarding loss of water by evaporation and with fæces in *Dixippus morosus* during its development from the third instar to the adult.

METHOD.

Nymphs of *Dixippus morosus* were reared in individual cages from the third instar or fourth instar to adults at 23° C. Two sets of three individuals were used, one at relative humidity 40 per cent., over calcium chloride, and the other in "saturated" air, over water. Although a hair hygrometer registered 100 per cent. humidity it seems unlikely that the air was really saturated since the glass tank in which the insects were kept was covered with a glass top raised $\frac{1}{4}$ of an inch above the sides. The insects continued to lose some water by evaporation in this atmosphere.

Mellanby (1932) has indicated the difficulty of obtaining true saturation. The insects were fed on fresh *Tradescantia* leaves.

Dixippus feeds heavily at night and very little during the day. Accordingly, the animals were taken from the leaves early in the morning, weighed, and placed in clean glass vessels without food or water. After nine hours they were again weighed and then placed on leaves so that they might feed again during the night. This process involves little interference with the normal life of the insect.

The fæces (if any) which were passed during the nine-hour period were carefully collected, dried to constant weight over sulphuric acid and weighed.

All weighings were made on a chainomatic balance accurate to 0.1 milligram.

The original weight minus the weight at the end of nine hours gives the total loss; and subtracting from this the weight of the dry fæces, gives the loss due to evaporation and water lost with the fæces. Given a value for evaporation, it is then possible to calculate the amount of water lost with the fæces.

This reasoning assumes that loss of weight is entirely due to evaporation and defæcation, and that the weights involved in the respiratory exchange may be overlooked. This assumption requires some examination.

Mellanby (1932), Gunn (1933), Koidsumi (1934), Buxton (1930), and Ramsay (1935) have shown from a wide variety of insect material that, provided the insect does not eat or produce fæces, loss of weight may be used as a satisfactory measure of loss of water by evaporation. This method was used by Wigglesworth and Gillett (1936) to estimate the amount of water lost from *Rhodnius prolixus* at ecdysis. Buxton (1930) has estimated the weight of carbon lost by respiration from starving meal-worms and shown it to be small enough to be neglected in most cases.

Gunn (1933) has discussed the factors involved in the change of weight of a starving insect. If the insect is allowed to take neither food nor water, the change in weight may be expressed as:

$$W_1 + aO_2 = W_2 + bCO_2 + cH_2O + \text{fæces.} \quad (1)$$

W_1 and W_2 are the weights of the animal before and after a certain period, and a , b , and c are the weights of the respective gases inspired or expired during this period. Expressing the respiratory quotient (R.Q.) in terms of weight, the following equation may be derived from (1):—

$$W_1 - W_2 = aO_2 \left(\frac{1}{8} \text{ R.Q.} - 1 \right) + cH_2O + \text{fæces.} \quad (2)$$

The expression $aO_2(\frac{1}{8} \text{ R.Q.} - 1)$ will represent the weight involved in the respiratory exchange. If the respiratory quotient is 0.73 or $\frac{1}{8}$, the value of this expression will be zero and respiration in itself will produce no change in weight. If the value of the respiratory quotient goes as high as 1.0, as it may in *Blatta orientalis* (Slater, 1927), then Gunn (1933) calculates that the upper limit of the change in weight due to respiration will be 10 per cent. of the total.

Dixippus morosus is a very sluggish insect, particularly during the day, when it will remain for long periods quite immobile. It is reasonable to assume therefore that its metabolism is low. In support of this is the observation that even a first instar nymph will live for more than two weeks at room temperature without food, provided the atmosphere is kept moist.

Buddenbrock and Rohr (1923) have published data for the respiration of adult *Dixippus*. From their figures the writer has calculated the respiratory quotient and the actual loss in weight due to respiration. At 23° C. for a period of 9 hours (the temperature and time interval of the experiments reported below) the loss is 0.85 milligram. Assuming the weight of an adult *Dixippus* to be 1 gram (a conservative estimate), the loss due to respiration during this period is 0.085 per cent. of the body weight. This is a negligible value. The present study is based, therefore, on equation (2) above, where $aO_2(\frac{1}{8} \text{ R.Q.} - 1)$ is negligible, and therefore:

$$W_1 - W_2 = cH_2O + \text{fæces.} \quad . \quad . \quad . \quad . \quad (3)$$

LOSS OF WATER FROM THE BODY SURFACE.

Water is lost from the body surface of insects mainly by evaporation from the spiracles; but it may also be lost from the general body surface (Ramsay, 1935). Buddenbrock and Rohr (1923) have shown for *Dixippus* that appreciable amounts of water are lost in this way. We are not here concerned with the site so much as the quantity of water-loss.

If it were possible to weigh the fæces accurately immediately upon release, then the loss in weight due to evaporation could be easily calculated from (3) above, as:

$$W_1 - W_2 - \text{fæces} = cH_2O. \quad . \quad . \quad . \quad . \quad (4)$$

However, due to the evaporation of water from the wet fæces and the minute weight of a single fæcal pellet, this is impracticable. An alternative method is to find a period when no fæces are lost.

Two and sometimes three days before ecdysis the nymphs of *Dixippus* cease feeding. After one or occasionally two days of fasting the gut is

almost emptied and defæcation stops until after the moult. During this period it is possible to get an accurate value for water lost by evaporation, since:

$$W_1 - W_2 = cH_2O. \quad (5)$$

This is the method used by Wigglesworth and Gillett (1936) to estimate the loss of water from *Rhodnius prolixus* at the last moult.

Figs. 1 and 2 show the absolute values for evaporation at the two humidities and the values relative to body weight. The weight of the

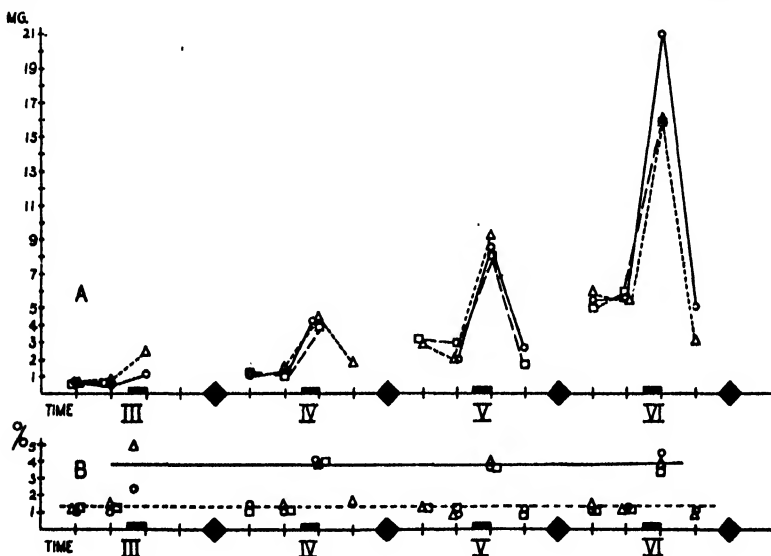


FIG. 1.—The loss of water at ecdysis for three individuals reared at 40 per cent. relative humidity.

A—The absolute loss. Ordinate: loss of weight in milligrams per nine hours. Abscissa: time-scale. Two intervals are equal to one day. The plotted points represent either the observed loss of weight during nine hours or the loss of weight in fifteen hours reduced to the equivalent value for nine hours. The solid rectangles on the abscissa mark the periods of actual moulting. The solid diamonds represent the periods between moults, and the roman numerals indicate the instars.

B—The loss relative to body weight. Ordinate: loss of weight expressed as a percentage of the body weight. Abscissa: as above. The broken line links the values calculated for the normal loss of weight and the solid line does the same for the increased loss at ecdysis.

exuvium has been subtracted from the loss at ecdysis, so that all values represent water loss only.

There is little or no increase in evaporation before the moult, although the old cuticle has been largely digested at this time and must be very thin. This supports the view of Kühnelt (1928) that the waterproof character of the cuticle resides in the epicuticle, and is in complete

agreement with the work of Wigglesworth and Gillett (1936) who give values for five days preceding the moult of *Rhodnius*.

At the time of actual ecdysis there is a sharp increase in evaporation amounting to about four times the "pre-moulting" value. This is true for every instar observed and for both humidities. Moulting occurs at night, and the next day evaporation has fallen to somewhere near the "pre-moulting" level. At the lower humidity evaporation falls to, or

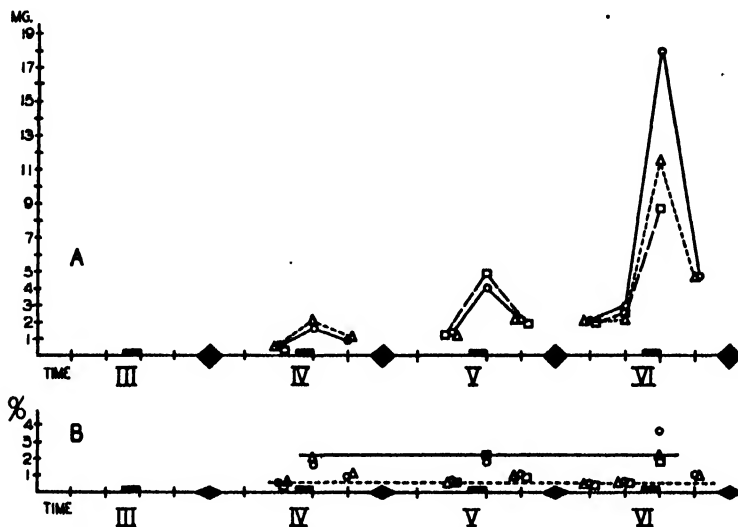


FIG. 2.—The loss of water at ecdysis for three individuals reared in "saturated" air.

A—The absolute loss.

B—The loss relative to body weight.

The ordinates and abscissæ are the same as those of fig. 1.

even below, the "pre-moulting" level; at the higher humidity evaporation falls to a point somewhat higher than the "pre-moulting" level on the day before ecdysis.

At the lower humidity evaporation in any given instar is about twice the evaporation at the higher humidity. This relationship holds for both the "pre-moulting" and the "moulting" levels of evaporation. The phenomena are therefore essentially parallel for both humidities.

From moult to moult the water-loss at both levels of evaporation increases by 2; and from moult to moult *Dixippus* increases its weight by approximately 2. At either level therefore evaporation maintains a constant relation to body weight, the loss by evaporation increasing in direct proportion to the increase in mass. In figs. 1 B and 2 B the evaporation is expressed as a percentage of the body weight, and the plotted points for the various instars fall on two straight lines corre-

sponding to the "pre-moulting" and the higher "moulting" levels of evaporation. There is no increase of body weight at the time of ecdysis. The "moulting" level of evaporation is accomplished therefore by an increased rate of evaporation per unit of mass; but at either level, from moult to moult, evaporation is maintained in linear proportion to mass.

These results will be further considered in the Discussion.

LOSS OF WATER FROM THE ALIMENTARY CANAL.

Water lost from the alimentary canal may be derived from the Malpighian tubules and the mid-intestine, both of which empty their products into the hind-intestine. Here, the rectal epithelium effects a partial, or sometimes total, absorption of water before the fæces are released (Wigglesworth, 1932). The amount of water lost from the alimentary canal is, therefore, the amount of water present in the fæces, and this amount will be a function of the absorptive activity of the rectal epithelium. Thus, the rectal epithelium appears to be the seat of an important part of the insect's water-conservation mechanism. Is its activity constant or does it vary with changes in development and environment?

As indicated above, it is impracticable to measure directly the amount of water in the fæces immediately upon their release. An indirect method must therefore be used.

From (3) above,

$$W_1 - W_2 = cH_2O + \text{fæces},$$

where W_1 and W_2 are the weights of the insect before and after a period of 9 hours and cH_2O is the weight of water lost by evaporation. "Fæces" as written here is composed of dry material (Fd) and water (Fw), and we may write:

$$W_1 - W_2 = cH_2O + Fd + Fw. \quad . \quad . \quad . \quad . \quad (6)$$

The weights of the insects before and after a period of 9 hours (W_1 and W_2) and the dry weight of the fæces lost during this period (Fd) are known. From (6), then, the actual weight of water lost by evaporation and in the fæces, is:

$$cH_2O + Fw = W_1 - W_2 - Fd. \quad . \quad . \quad . \quad . \quad (7)$$

Now, $\frac{W_1 - W_2 - Fd}{W_1 - W_2} \times 100$, will express the percentage of the total loss lost as water.

Fig. 3 A shows typical values of this ratio for the last four nymphal instars. It is apparent that most of the loss is due to loss of water. Moreover, the value of the ratio, in any instar, remains more or less

constant until just prior to the moult when it is sharply depressed. This means that just before the moult a smaller proportion of the loss in weight is attributable to water, or, conversely, a larger proportion is due to dry material. The water is lost by evaporation and with the fæces. Consequently, one or both of these components must decrease prior to ecdysis to account for the decrease of the ratio at this time. But we have shown above that evaporation increases with increasing body weight and will therefore have a higher value towards the end of the instar than

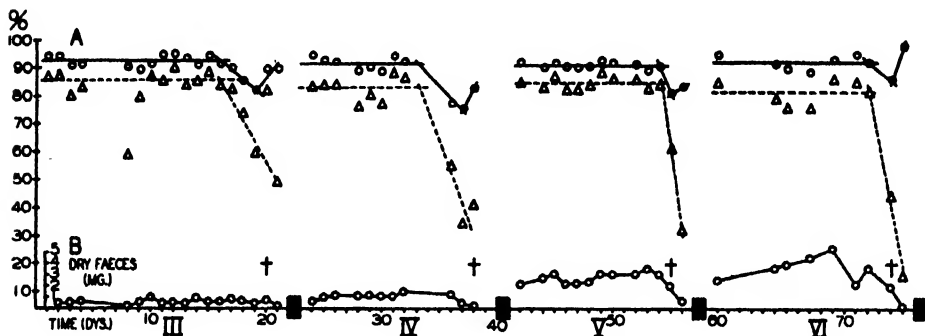


FIG. 3.—Data for a single individual showing typical values.

A—The circles and solid line indicate the values for the percentage of the total loss of weight (during nine hours) lost as water (water of evaporation plus water of fæces). The triangles and broken line indicate the values for the percentage of the total loss of weight lost as water with the fæces.

B—Ordinate: dry weight of fæces lost in nine hours. Ordinate marked in milligrams.

The abscissa is common to both graphs and shows the time in days. The solid rectangles mark the periods of ecdysis and the roman numerals denote the instars. The crosses indicate the cessation of feeding.

at the beginning. The decrease of the ratio must therefore be due to a smaller amount of water in the fæces. Thus it appears that, prior to the moult, the dry component (dry weight of fæces) of the loss in weight is accompanied by a smaller proportion of water. The insect might be said to prepare for the process of moulting, with its resultant loss of water (figs. 1 and 2), by retaining some of the water that is normally lost with the fæces.

The depression of the value of the ratio, "percentage of the total loss lost as water," is in every case followed by a rise in this value. This occurs on the last day of defæcation when the amount of fæces is very small (fig. 3 B), and may be explained as follows. When no fæces are lost, the percentage of the total loss lost as water will be 100 per cent. since evaporation accounts for the entire loss. Similarly, when the amount of fæces is very small, most of the loss will be due to evaporation,

and the ratio, "percentage of the total loss lost as water" will have a high value.

If now we exclude that part of the loss due to evaporation and express the ratio in terms of water lost only in the fæces, it should show a steadily decreasing value with no final upward tilt. From (7), the weight of water lost with the fæces will be

$$Fw = W_1 - W_2 - Fd - cH_2O. \quad (8)$$

A value for the weight of water lost by evaporation, (cH_2O), is available when defæcation ceases before ecdysis (figs. 1 and 2). W_1 , W_2 and Fd are known. Substituting these values in the above equation gives the weight of water lost with the fæces. The ratio, $\frac{W_1 - W_2 - Fd - cH_2O}{W_1 - W_2} \times 100$, will now express the percentage of the total loss lost as water in the fæces.

The values for this ratio are plotted on fig. 3 A (broken line and triangles). The points fluctuate rather widely between 70 per cent. and 90 per cent. until just before ecdysis when there is a sharp, uninterrupted decrease. The very low value on the last day of defæcation is to be expected since at this time a large part of the total loss is due to evaporation, and the small amount of fæces implies a small value for the percentage of the total loss lost as water in the fæces. However, the preceding low value of the ratio is accompanied by no significant change from the normal amount of fæces (fig. 3 B) and we must conclude that just before the moult the fæces are eliminated with less water than at any other time during the instar.

A further consideration of the amount of water in the fæces emphasizes this conclusion. It has been shown above, (8), that the weight of water lost with the fæces may be expressed as

$$Fw = W_1 - W_2 - Fd - cH_2O.$$

From this, the total weight of the fæces (dry material plus water) will be

$$Fd + Fw = W_1 - W_2 - cH_2O. \quad (9)$$

The ratio, $\frac{W_1 - W_2 - Fd - cH_2O}{W_1 - W_2 - cH_2O} \times 100$, will then express the percentage of water in the fæces.

Fig. 4 presents in graphic form the values of this ratio for the six individuals used in this study. Just before the moult the percentage of water in the fæces is considerably less than at any other time during any instar. The cessation of feeding always occurs after the initial and usually after the final decrease of the ratio, "percentage of water in the

fæces." The final decrease is often followed by a slight rise in the value of the ratio, and this rise corresponds to the last day of defæcation. There is a tendency for those insects reared at the lower humidity to eliminate drier fæces just before the moult than those reared at the higher humidity.

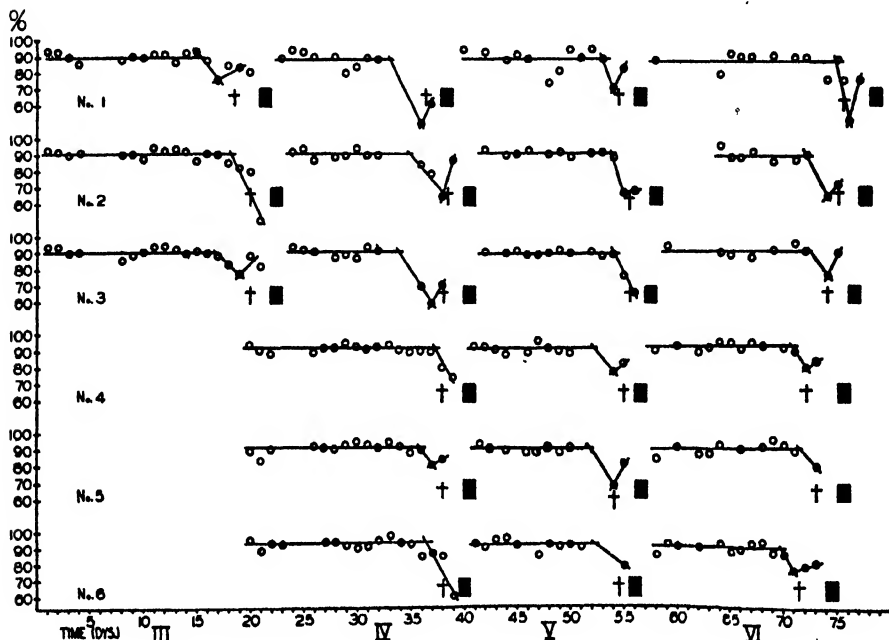


FIG. 4.—The percentage of water in the fæces. The data for the six individuals are plotted on six separate ordinates, the ordinates showing the percentage of water in the fæces. The abscissa is common to all the data and shows the time in days. The roman numerals indicate the instars. The solid rectangles mark the periods of ecdysis and the crosses indicate the times at which feeding ceased.

Numbers 1, 2, and 3 were reared at 40 per cent. relative humidity. Numbers 4, 5, and 6 were reared in "saturated" air.

DISCUSSION.

Wigglesworth and Gillett (1936) have shown for *Rhodnius prolixus* that there is no increase in evaporation before the moult; but at the time of moulting evaporation is about doubled. Figs. 1 A and 2 A of the present study indicate that for *Dixippus morosus* evaporation is increased roughly four times at ecdysis. This is true for all instars examined and at both the high and low humidities. Moreover, the "normal" (*i.e.* pre-moulting) amount of water lost by evaporation is greater for *Dixippus* than for *Rhodnius*. At 0 per cent. relative humidity and 24° C. *Rhodnius* loses about 1.5 per cent. of the body weight per diem, while at 40 per cent. humidity and 23° C. *Dixippus* loses about 3.1 per cent. per diem.

Both the "normal" loss and the extra loss at moulting are therefore at a higher level in *Dixippus* than in *Rhodnius*. It is suggested that this difference is related to the difference in the normal habit of these two insects, *Rhodnius* living in an arid environment while *Dixippus* lives and feeds on more or less lush vegetation.

As in *Rhodnius*, *Dixippus* shows no marked increase in evaporation before the moult, although the old cuticle must be very thin at this time. As pointed out by Wigglesworth and Gillett (1936), "That supports the view that the waterproof properties of the insect cuticle reside in the outermost, non-chitinous, layer—the epicuticle (Kühnelt, 1928)."

Although *Rhodnius* loses less water than *Dixippus* during actual ecdysis, this loss remains above the "normal" level for about 5 days, while the evaporation from *Dixippus* apparently returns to "normal" within about 1 day. In fact, the loss from *Rhodnius* on the day after ecdysis is very little less than the loss on the day of ecdysis, and decreases gradually until a constant level is reached on the fifth day. Feeding occurs after the eighth day. This whole period is greatly abbreviated in *Dixippus*. Ecdysis, with the associated high loss of water by evaporation, takes place at night. The following day evaporation has fallen to about the "normal" level, and feeding usually begins during the ensuing night. At the higher humidity (fig. 2 A) on the day after ecdysis, evaporation is sharply reduced from the "moulting" level to a value somewhat above the "normal." At the lower humidity (fig. 1 A) the evaporation on the day after ecdysis actually falls below this "normal." Thus it seems that although *Dixippus* loses more water than *Rhodnius* during actual ecdysis, this loss is effective for a much shorter period, and the total loss associated with moulting will be about the same for both insects.

It follows from the above that, whatever property is responsible for the reduction of evaporation after the moult, its action is more rapid in *Dixippus* than in *Rhodnius*. The literature contains two references pertinent to this property. Observing a gradual decrease in the evaporation from newly moulted caterpillars, Koidsumi (1934) related this in part to the gradual thickening of the cuticle. From their observations on *Rhodnius*, Wigglesworth and Gillett (1936) conclude: "But in view of the fact that the endocuticle is certainly permeable to water, we think it more probable that the progressive loss of permeability in *Rhodnius* is due to the hardening of the cuticulin in the outer layers of the cuticle. Our main conclusion, however, is that the impermeability of the cuticle is very nearly established *before* the old skin is shed, so that the extra loss of water associated with moulting is very small." If this is the case we

must conclude that the impermeability of the epicuticle of *Dixippus* is established much more quickly than in the case of *Rhodnius*. The greater loss during actual ecdysis is thus offset to ensure for *Dixippus* a minimal loss of water at this critical period.

At the higher humidity (fig. 2 A) the rate of evaporation is greater after ecdysis than before it, while at the lower humidity (fig. 1 A) the rate of evaporation after ecdysis is about the same or slightly less than before. With the spiracles opened by exposure to dry air containing 10 per cent. CO₂, adults of *Rhodnius prolixus*, seven days after ecdysis, lost three times as much water as the preceding nymphs; in dry air alone the adults lost slightly less water seven days after ecdysis than before it (Wigglesworth and Gillett, 1936). This parallel suggests that moist air and CO₂ have the same effect on evaporation. The effect of CO₂ is to open the spiracles, and Mellanby (1932) attributes the same effect to moist air. Hazelhoff (quoted by Jordan, 1927) has demonstrated that the snail *Helix* closes the pulmonary aperture most of the time in dry air but opens it in saturated air.

At ecdysis the evaporating surface of the tracheæ and the spiracular apertures are enlarged with the increase in general size so that if the spiracles are kept more or less open by exposure to moist air, or CO₂, evaporation will be greater after the moult than before it. This is the explanation advanced by Wigglesworth and Gillett (1936) for *Rhodnius* in air containing 10 per cent. CO₂, and it seems to fit equally well the similar results obtained with *Dixippus* in moist air. In *Dixippus*, where the observations were made on the day after ecdysis, an additional factor may be the permeability of the tracheal cuticle immediately after ecdysis. The ultimate reduction of this permeability with increasing age (Koidsumi, 1934; Wigglesworth and Gillett, 1936) will lead to a lower level of evaporation at the higher humidity than that observed on the day after ecdysis.

Since their observations were confined to a single moult, Wigglesworth and Gillett (1936) were unable to show the linear relation between evaporation and body weight which has been demonstrated above (figs. 1 B and 2 B). Gunn (1935) presents data which show that, for a difference of almost 600 mg. in weight, the cockroaches *Periplaneta americana* and *Blatta orientalis* exhibit a close proportion between evaporation and body weight (in dry air at 20° C.). We have seen that, from the third moult (body weight 48 mg.) to the sixth moult (body weight 450 mg.), *Dixippus* shows a similar proportion between evaporation and body weight at the time of ecdysis.

Observing that the very small cockroach *Blatella germanica* loses water at a higher rate than the two larger species, Gunn (1935) states:

"This suggests that the water vapour evaporates through the surface of the animal, since if the shape remains constant the smaller the animal the larger the surface area per gram." However, on the basis that surface area is proportional to body weight $2/3$, he calculates the water-loss per square centimetre and shows that it is not constant but decreases from the largest to the smallest species. The same calculation gives a similar result for *Dixippus*, the water-loss per square centimetre of body surface increasing from the third moult to the imaginal moult. In a developing insect there can be no constant relation between surface area and weight, since the surface area remains more or less the same throughout any instar while the weight increases. It seems possible, however, that just before the moult the weight will be in equilibrium with the surface and the relation, Surface Area = K Body Weight $2/3$, will be constant in any instar. An investigation of this point should be of interest.

The work of Ramsay (1935) has shown that the results of experiments on the evaporation from insects must be interpreted with caution. He has shown that the evaporation from *Periplaneta americana* takes place principally from the tracheal system; but considerable amounts of water are lost from the general body surface. The latter appears to be more complicated than the evaporation from the tracheal system which may be adequately explained on a physical basis. Koidsumi (1934) and Buddenbrock and Rhor (1922) have also shown that a part of the water loss of insects may take place through the general body surface. Koidsumi (1934) has further shown that the evaporation from the body surface of *Milionia* pupæ does not conform with the physical law of evaporation from a simple water surface.

"Evaporation" as considered in this paper includes loss of water from both the general body surface and the tracheal system. It is not likely therefore that we will be able to draw any conclusions as to the site of the loss. However, the observed proportion between evaporation and body weight at the time of moulting may be of significance to the study of evaporation from insects.

Wigglesworth and Gillett (1936) have shown that, in dry air containing 10 per cent. CO_2 , the spiracles of *Rhodnius prolixus* are opened and the evaporation is enormously increased. It is apparent, then, that considerable quantities of water are lost from the insect when the spiracles are open; and Mellanby (1934) concludes that the greater part of the evaporation from insects takes place in this way. The spiracles are normally opened to supply the needs of the respiratory system (Hazelhoff, 1927). Gunn (1933) gives evidence that, under the same humidity conditions and within certain temperature limits, evaporation will be proportional

to the degree and extent of opening of the spiracles, and hence to the rate of respiration. A small proportion of the respiratory exchange may take place through the integument (Buddenbrock and Rohr, 1922; Fraenkel and Herford, 1938). Gunn (1935) adds further weight to this conclusion by showing that in three species of cockroach evaporation is proportional to respiration, 6 mg. of water being lost for every 1 mg. of oxygen consumed.

In the case of *Dixippus*, evaporation is proportional to the mass of the insect at the time of the third, fourth, fifth, and sixth moult (figs. 1 B and 2 B). If, then, evaporation is proportional to respiration and water is lost only from the tracheal system, we must conclude that, from the third instar to the imago, respiration is proportional to mass at the time of ecdysis. This appears to be the case in *Periplaneta orientalis* where, from body weight 200 mg. to body weight 900 mg., respiration remains proportional to mass (David and Slater, 1926). However, the work of Bodine (1921) and Butler and Innes (1936) indicates that the respiration of grasshoppers is proportional to a fractional exponent of the body weight. Gunn (1935) shows that the respiration in three species of cockroach is proportional to body weight to the power of 0.75-0.8. It seems doubtful, therefore, if the observed relation between evaporation and the mass of *Dixippus* is a simple function of the respiration. In so far as evaporation takes place from the tracheal system it is probably proportional, at constant humidity, to respiration. In addition, some evaporation takes place from the body surface and this is complicated by an hydrophilous film and the possibility that water is supplied to the surface at a limited rate (Ramsay, 1935). There is also the possibility that the permeability of the cuticle changes in a developing insect. We must therefore conclude that evaporation from insects is a heterogeneous physical system having two components, evaporation from the tracheæ and evaporation from the general body surface. In *Dixippus* these two components are so combined that the total evaporation is proportional to the weight of the insect at ecdysis.

The "moulting" level of evaporation from *Dixippus* offers further difficulties of interpretation. For if the increased loss of water at ecdysis is due to increased permeability of the cuticle, as suggested by Wigglesworth and Gillett (1936), it should be proportional to the surface area of the insect (body weight $2/3$). Figs. 1 B and 2 B, however, indicate that the "moulting" level of evaporation exhibits the same linear proportion to body weight as does the lower "pre-moulting" level. This suggests that during ecdysis water is lost in essentially the same manner as before, although at a higher rate. In other words, if the permeability of the

cuticle is actually increased at this time, then the amount of water lost from the tracheæ must be proportionately increased; and, since all the available evidence indicates that the greater part of the water-loss from insects takes place from the tracheæ and not through the cuticle, this will also be true at the time of ecdysis.

The insect undoubtedly performs considerable work in freeing itself from the old cuticle. The resultant increased metabolism, necessitating a higher rate of respiration, will produce a proportional increase in evaporation. Furthermore, if the new cuticle is more or less permeable to water immediately after ecdysis, then the cuticle of the trachea will be likewise permeable. Wigglesworth (1938) has shown that the trachea of the mosquito, *Aedes aegypti*, remain permeable to water for a few minutes after moulting, during which time water is actually absorbed from them and is replaced by air *via* the respiratory siphon.

Under similar conditions of humidity, therefore, the insect will have a much higher rate of evaporation at ecdysis due to increased respiration and permeability of the cuticle; but this evaporation will have the same relation to respiration and evaporation through the body surface as before ecdysis.

The second part of this paper deals with the loss of water from the alimentary canal, and from the results presented we were led to conclude that immediately prior to ecdysis the fæces were eliminated with less water than at any other time during the instar. In the absence of comparable work, the discussion will be limited.

The main assumption involved in the calculations supporting this conclusion is that the evaporation is constant in any instar and the value available when no fæces are lost, preceding ecdysis, may be applied throughout the instar. In the preceding discussion it has been shown that evaporation in *Dixippus* is related to body weight at ecdysis. If this relation holds, not only at ecdysis, but during the instar, then evaporation will not be constant throughout the instar but will increase with increasing weight. However, in the absence of data for evaporation throughout the instar we have used the value available at the end of each instar.

The part played by evaporation is, in any case, small. When no fæces are lost and the total loss in weight is due to evaporation, this loss represents, at the most, a little more than 1 per cent. of the body weight; but when defæcation occurs the loss of water may be as much as 20 per cent. of the body weight. Moreover, if the evaporation were large and increased with increasing weight there should be an increase in the percentage of the total lost as water instead of the observed decrease at the end of any instar (fig. 3). It appears, therefore, that the fæces

account for the greater part of the loss in weight, and any decrease of the percentage of water in this loss will be due to less water in the fæces, since the only other component of the loss is evaporation, which is small and will tend to increase throughout the instar.

It is of interest to consider how a reduction of the percentage of water in the fæces might be accomplished. There is no indication that it is related to the cessation of feeding before ecdysis, since feeding stops after the initial, and usually after the final, decrease of the percentage of water in the fæces (fig. 4). During the period when no food is taken (and hence, no water is gained) the insect continues to lose water by evaporation and some by defæcation. The reduction in the amount of water lost with the fæces might, therefore, be regarded as an adaptation to offset the loss of water associated with ecdysis. Any undue loss of water at this time would be deleterious to moulting, since water is lost mainly from the blood (Mellanby, 1939) and the maintenance of blood volume is necessary for the mechanical operation of rupturing and escaping from the old cuticle. Mellanby (1939) has pointed out that in insects exposed to desiccation for some time the blood is reduced in volume; and since the blood volume has important mechanical functions to perform in hatching and moulting these processes are adversely effected. Thus, pupæ of tsetse flies, though completely developed, may be unable to escape from the puparium.

Wigglesworth (1932) has shown that the rectal epithelium or "rectal glands" absorb water from the fæcal matter in the rectum before the fæces are released; and, as we have stated above, the amount of water lost with the fæces will, therefore, be a function of the absorptive activity of the rectal epithelium. It follows, then, that any decrease of the amount of water in the fæces will be due to an increased absorption by the rectal epithelium. We therefore conclude that, just before the moult, the rectal epithelium absorbs more water from the fæces than at any other time during the instar.

This conclusion suggests an interesting terrestrial parallel to a similar phenomenon reported by Maluf (1939) for certain marine crustaceans. Evidence from blood studies indicates that preceding the moult these crustaceans actively absorb water from their environment, and Maluf states: "The imbibition of water increases the blood pressure and thus aids ecdysis and the expansion of the new integument." The increased absorption of water by *Dixippus* may not be sufficient to increase the blood volume, but it may well serve to protect it against undue loss by evaporation in the absence of feeding, and thus to aid ecdysis.

The increased absorption of water from the fæces is associated with

moulting; and moulting is controlled by a hormone (Wigglesworth, 1934). We might consider, then, that the increased absorptive activity of the rectal epithelium is due to an accessory action of the moulting hormone. The moulting hormone is no doubt responsible for the secretion of the moulting fluid and its ultimate reabsorption. Wigglesworth (1933 *b*) has shown that 86 per cent. of the abdominal cuticle of *Rhodnius prolixus* is digested by the moulting fluid and reabsorbed through the general body surface. The cuticle of the proctodæum will be digested and absorbed in a similar manner. The digestion of the lower layers of the proctodæum cuticle by the moulting fluid will increase its osmotic pressure and tend to increase the absorption of water from the rectum. At the same time considerable osmotic forces must be exercised to effect the reabsorption of the moulting fluid and the products of its digestion. It is suggested that these forces, acting secondarily on the contents of the rectum, will effect an increased absorption of water and thus account for the observed decrease of the percentage of water in the fæces just before ecdysis. If, further, the impermeability of the new cuticle is almost established before ecdysis, as suggested by Wigglesworth and Gillett (1936), then absorption will be reduced and this may account for the increase of the percentage of water in the fæces frequently observed on the last day that fæces are lost before ecdysis (fig. 4).

This research was carried out at the Department of Zoology in the University of Edinburgh. I wish to express my gratitude to Professor James Ritchie for facilities provided and for his kindly advice and criticism. To Dr F. Gross of the same department I am indebted for many suggestions and constant encouragement. Professor N. J. Berrill has offered me hospitality at the Department of Zoology, McGill University, where this paper was written.

SUMMARY.

1. Data are presented for the loss of water by evaporation and with the fæces of *Dixippus morosus* during its development from the third or fourth instar to the adult. Water lost by evaporation is measured directly during the period of ecdysis; water lost with the fæces is estimated by an indirect method.

2. The evaporation remains relatively constant before ecdysis; but during ecdysis it is increased roughly four times. On the day after moulting, evaporation decreases to about the "pre-moulting" level.

3. These results are compared with the results of a similar study by Wigglesworth and Gillett (1936) using the insect *Rhodnius prolixus*. The results are similar in form but different in detail.

4. The rate of loss of water is greater at the lower humidity but essentially parallel to the loss at the higher humidity. There is some evidence that the spiracles are opened more in moist air than in dry air.

5. From moult to moult, the normal evaporation, as measured preceding ecdysis, is maintained in linear proportion to the body weight. This proportion remains unexplained, but respiration is indicated as the chief agent by which it is effected.

6. From moult to moult, the extra evaporation associated with actual ecdysis is also maintained in linear proportion to body weight. It is suggested, therefore, that evaporation is accomplished at a higher rate but in the same way at ecdysis as before ecdysis. A plausible explanation, involving increased metabolism and increased permeability of the tracheal cuticula at ecdysis, is outlined.

7. When faeces are lost, the greater part of the loss in weight is due to water lost with the faeces.

8. In any instar and at both the high and low relative humidities, the percentage of water lost with the faeces remains practically constant until just before the moult when it is sharply decreased.

9. The decrease of the percentage of water in the faeces is ascribed to an increased absorption of water by the rectal epithelium; and this in turn may be related as an accessory action to the absorption of the moulting fluid and the products of its digestion before ecdysis.

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XV.—A Simple Apparatus for the Direct Determination of the Volume of Small Irregular Objects. By **Beverley N. Smallman, M.A., Ph.D.**, Department of Zoology, McGill University.* *Communicated by Professor JAMES RITCHIE, M.A., D.Sc.* (With One Text-figure.)

(MS. received June 2, 1941. Read July 7, 1941.)

THE apparatus described was developed in connection with a study of the growth and development of the stick insect, *Dixippus morosus*. The

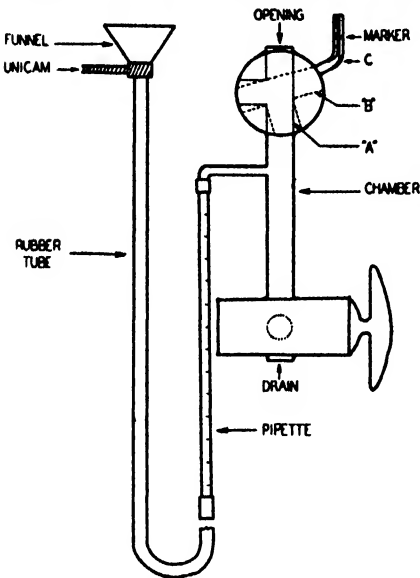


FIG. 1.—The apparatus. The solid line, "A," indicates position "A" referred to in the text; the dotted line, "B," indicates position "B."

principle is that of simple displacement, and consistent results are obtained with objects as small as 0.007 millilitre.

The apparatus is shown in fig. 1. It consists of two vacuum stop-cocks joined by a tube which serves as a specimen chamber. The top stop-cock is of the 3-way type and has a side-tube (C) of 1 millimetre bore fused to the upper part of the wall. The lower stop-cock serves merely to drain the vessel. A side-arm is fused to the specimen chamber and a graduated pipette is attached to this by means of heavy rubber tubing. The pipette is continuous with a rubber tube and funnel supported by the arm of a unicam. The stop-cocks were lubricated with Apiezon "L" stop-cock grease.

Mercury is introduced into the funnel and rises into the pipette until it reaches the level of the side-arm of the specimen chamber. With the stop-cock in position "A," water is introduced into the specimen chamber until it flows into the side arm and comes into direct contact with the

* The work reported in this paper was carried out at the Department of Zoology, University of Edinburgh.

mercury. More water is added until it rises into the bore of the stop-cock. The mercury column is then lowered until the mercury-water partition is near the lower end of the pipette. The stop-cock is now turned to position "B."

The vessel now forms a continuous fluid system and the only opening is the tube C. Raising the mercury in the pipette forces the water into the tube C where it is brought to coincide with the reference level marker. The height of the mercury in the pipette is read, and this reading is the zero for the subsequent measurement.

The mercury column is now lowered until the water level falls from tube C into the bore of the stop-cock where an air-space is formed. This air-space should be somewhat larger than the size of the object to be measured. The stop-cock is now returned to position "A" and the air-space rises in the bore so that the level of the water is somewhat below the opening. The object is now inserted at the opening and sinks into the specimen chamber. The stop-cock is again turned to position "B" and the mercury column raised until the water level once more coincides with the reference level. The pipette is now read and the difference between this reading and the former reading gives the volume directly.

The volumes of small strips of copper of known weight were calculated from the specific gravity. Their volume was then measured with the described apparatus, using a pipette graduated in intervals of 0.002 millilitre. The following results were obtained. For convenience, the results are expressed in cubic millimetres.

Calculated Volume, cu. mm.	Observed Volume (Mean of 10 Measurements), cu. mm.	Standard Deviation of the Mean.	Coefficient of Variation.
7.0	6.75	± 0.52	7.7%
10.5	10.40	± 0.46	4.6%
28.7	27.80	± 0.74	2.7%
61.7	59.90	± 1.10	1.8%

SUMMARY.

An apparatus for the direct determination of the volume of small irregular objects is described. The principle is that of simple displacement. Results are presented which indicate that consistent results may be obtained with objects as small as 0.007 millilitre.

This work was carried out at the Department of Zoology in the University of Edinburgh. The writer is grateful to Professor James Ritchie and Dr F. Gross for much help and encouragement.

(Issued separately January 30, 1942.)

XVI.—Observations on Artificially Induced Heat in Immature Guinea-Pigs. By **P. Bacsich**, M.D., Carnegie Teaching Fellow, and **G. M. Wyburn**, M.B., Ch.B., D.Sc. From the Department of Anatomy, University of Glasgow. (With Two Plates.)

(MS. received June 10, 1941. Revised MS. received September 23, 1941.

Read July 7, 1941.)

TREATMENT of the immature animal with oestrogenic or gonadotropic substances has been described by a number of authors in diverse experiments, and vaginal cornification, enlargement and typical histological changes in the uterus, ovulation, mating, and fertilisation, have all been produced, but no successful implantation. The histological changes which occur in the uterus and vagina of the treated immature animal are considered (Reynolds and others) to be characteristic of heat and similar to those in the adult uterus during heat. Nevertheless, it is suggested that the failure to procure implantation in treated immature animals may be due to an unfavourable condition of the uterine endometrium. If that is so, there must exist some difference between the response of the treated immature uterus and that of the mature uterus during oestrus which has thus far escaped detection. Recently we have described (Bacsich and Wyburn, 1940 *b*) a cyclic vascular change in the uterus of the guinea-pig in the form of antimesometric hyperæmia present only during normal heat, and, moreover, have been able to induce this vascular response in spayed animals by hormonal treatment (Bacsich and Wyburn, 1941). As this cyclic vascular change provides a particularly delicate test of a complete response of the uterus to ovarian hormonal activity and may influence the site and the course of implantation, it seemed of interest to ascertain if the "characteristic" oestrogenic response of the immature uterus included antimesometric hyperæmia.

MATERIAL AND METHODS.

Fifteen immature female guinea-pigs of ages ranging from 10 to 12 weeks were used in the course of the experiments. The animals were obtained at an early age from a reliable dealer—our regular source of supply—and were kept and observed for some time before treatment.

In all the animals the external genitalia were undeveloped, the vaginae firmly closed, and in no case was there any sign of spontaneous heat. Three animals were used as controls and later killed. The remaining twelve were given injections of œstradiol monobenzoate (Progynon B oleosum forte) and progesterone (Proluton) as follows:—

- (1) Priming. Daily injections of five units of œstrin (0.0005 mg.) until the vaginae were definitely open, in from 5 to 8 days.
- (2) Massive doses. 3000 units (0.3 mg.) of œstrin on two consecutive days.
- (3) Progesterone. 0.1 mg. of progesterone on the day following the second large dose of œstrin.

This was the method of treatment which in a previous work (Bacsich and Wyburn, 1941, Exp. 5) was found to give consistently the best result. The massive doses of œstrin, however, have been reduced from 5000 to 3000 units in proportion to the weight of the animals, and the Proluton from 0.2 mg. to 0.1 mg. After the vaginae opened smears were taken and examined (see method in Bacsich and Wyburn, 1940 *a*) at frequent intervals, including one before and one 3–4 hours after progesterone administration (the latter just prior to death). The animals were killed 3–4 hours after the injection of progesterone and the control animals were killed simultaneously. At autopsy the ovaries were observed and attention directed to the appearance of the uterus. For the study of vascular response the uteri were removed, fixed, sectioned at 100 μ and stained by the benzidine reaction as already described (Bacsich and Wyburn, 1940 *b*). In each case some sections of the uterus were also cut at 10 μ , stained with hæmalum and eosin, and the histological changes observed.

DESCRIPTION.

Control Animals Nos. 1, 2, and 3.

Macroscopic observations: Ovaries small, no follicles or corpora lutea; uteri very small, almost thread-like in appearance, anæmic; vaginae closed.

Microscopic examination: Here our attention was confined to sections of the uterus.

Histological conditions: Low cubical epithelium; glands few, straight and unbranched; small stroma cells; muscular layers relatively well developed. Vascular pattern (Pl. I, fig. 1)—an equal distribution of radially arranged blood-vessels forming a fairly close regular network in the endometrium terminating in a subepithelial loop formation and a

more open and irregular network in the circular layer of the myometrium. There were comparatively few blood-vessels in the longitudinal coat of myometrium and these were parallel with the course of the muscle fibres.

Treated Animals Nos. 4-15.

Macroscopic observations: Ovaries small; as in the control animals, there were no follicles or corpora lutea. The uteri were very much enlarged, approximating to and often attaining the size of the adult uterus on heat. An exception is animal No. 7, where the uterus showed only moderate increase in size. Vascularity was variable. In three animals (Nos. 7, 12, 15) uteri were relatively anæmic, but in the remaining nine they were markedly hyperæmic, and in animals Nos. 10 and 11 the appearance was almost cyanotic. The segmental distribution of the arteries was particularly prominent in animal No. 9.

In the majority of animals (No. 15 in particular) there was a rather striking swollen œdematous appearance of the uteri, much more apparent than during normal œstrus, or in the uteri of spayed adults after artificially induced heat.

Vaginæ were enlarged and well open. A careful examination and comparison of successive smears yielded some evidence of progressive change, but there were no clear-cut stages each with its own characteristic cell type. Leucocytes, nucleated cells, cornified and deep epithelial cells were present in all the vaginal smears from the time the vagina opened until the death of the animal. Only the preponderance of one or other type of cell in any one smear afforded a possible clue to what could at best be but an arbitrary division into stages of heat. This indeterminate nature of the cellular picture of the vaginal smear might well indicate a discordant vaginal reaction---different parts of the vagina reacting with different speed and varying intensity. It is of interest that animal No. 7, with relatively little uterine reaction, exhibited as good a vaginal reaction as the other treated animals.

Microscopic examination of uterus:

Histological observations: The following description applies to all the treated animals except No. 7, in which the findings were very largely negative. The uterine epithelium was low columnar as compared with the cubical epithelium of the immature uterus. Glands were numerous and enlarged with much branching. The neck of the glands displayed numerous mitotic divisions. Endometrial response was well marked, with large stroma cells with deeply stained nuclei. There was a considerable increase in the myometrium, particularly in the circular layer.

This histological picture shows little if any difference from the usual

description of the normal uterus during heat, and judged by that standard the immature uterus has been successfully converted into a sexually mature condition. It should, however, be noted that in two of our animals (Nos. 8, 14) a closer examination of serial sections revealed that the histological changes were not constant throughout the sexually active portion of the uterus, but that there were parts with little or no reaction.

Vascular changes: With the exception of animals Nos. 7, 12, and especially 15, where the response was on a very limited scale, sections of the uteri of the other animals stained by the benzidine reaction showed an increased, but more or less uniform generalised vascularity (Pl. I, fig. 2). In no case was there present the definite antimesometric hyperæmia which occurs during normal heat or induced heat in spayed adult animals.

In some animals (Nos. 9, 11) there was an attempt to produce antimesometric hyperæmia (Pl. I, fig. 3), but this was confined to a few sections and did not involve the whole of the sexually active part of the uterus. In animal No. 10 a crude effort has resulted in some subepithelial hæmorrhages in the antimesometric region (Pl. I, fig. 4), again limited to a few sections, while in other cases (animals Nos. 5, 13) the specialised vascular response, although evident on the two sides of the antimesometric half of the uterus, is absent from the antimesometric pole (Pl. II, fig. 5). There were also uteri (animals Nos. 4, 8) with a localised hyperæmia on one or other side (Pl. II, fig. 6) or even at the mesometric corner. Animal No. 14 (Pl. II, fig. 7) was a good example of a rather interesting type of reaction—one side of the uterus displayed an energetic endometrial and vascular response, but on the other side there was no sign of either endometrial or vascular activity.

DISCUSSION.

In a former work (Bacsich and Wyburn, 1941, Exp. 5) we produced by hormonal treatment a characteristic antimesometric hyperæmia in 7 out of 10 spayed guinea-pigs and concluded that this vascular reaction in the normal animal, like other œstrous changes, is controlled by the activity of the ovarian hormones. It was, however, emphasised that whereas some degree of uterine response such as endometrial proliferation may be expected from almost any form of œstrogenic stimulation, a more subtle approach, including preliminary priming with small doses of œstrin, was essential to achieve the hormonal balance necessary to elicit with any consistency the specialised and more delicate vascular response. In the present investigation twelve immature animals were subjected to the same form of hormonal treatment, which in 70 per cent. of spayed adults was followed by a successful demonstration of anti-

mesometric hyperæmia. In not one of these immature animals was the characteristic antimesometric hyperæmia developed, although in other respects—growth and distension of the uterus, proliferation of endometrium, histological changes in uterine epithelium and glands—there was abundant evidence of hormonal activity. In most of the treated animals there is an undoubted vascular reaction as evidenced by a general hyperæmia. Furthermore, there may be a haphazard localised vascularity confined to patches of the sexually active part of the uterus almost as though the uterus were eager to respond in normal fashion, but the efforts are incomplete or misdirected. The same type of patchy or mosaic response is shown in an unequal endometrial proliferation, some parts reacting more vigorously than others. This suggests a lack of effective co-ordinated action.

The indeterminate nature of the vaginal smears—there is no clear-cut indication of the recognised stages of heat—might also be interpreted as implying lack of uniformity in the proliferation of the vaginal epithelium, a sluggish or incomplete reaction at one point contrasting with a more vehement activity elsewhere.

While therefore as assessed on the basis of increase in size and general endometrial proliferation we agree with Levin and Tyndale (1937) and Freed and Soskin (1937) that in the immature uterus, as quoted by Reynolds, "the somatic substrate is receptive to the ovarian hormones," the further suggestion of the above authors, again quoted by Reynolds, that "the immature uterus responds so sensitively and characteristically that it is recommended as a test-object for the biological assay of gonadotropic and œstrogenic preparations," requires, in view of our results, careful consideration. We have to admit that the test based on the increase in weight of the uterus gives some useful information in that it demonstrates that the substance tested has some œstrogenic capacity. But would this mere increase in weight of the immature uterus offer any guarantee that the substance tested is capable of eliciting all the physiological heat changes (antimesometric hyperæmia included) of the adult uterus? Our findings lead us to conclude that the response of the immature uterus to œstrogenic stimulation when compared with that of the adult uterus is qualitatively deficient, and is thus incapable of furnishing information regarding the total efficiency of the substance so tested.

Mirskaya and Crew (1930 *a*) provoked "œstrus" in immature mice by the injection of urine from pregnant women, and while in some instances successful mating occurred, in no case did pregnancy follow coitus. They express the view that the cause of infertility was the lack

of preparedness on the part of the uterus to receive the egg. Engle (1931) treated a group of immature mice with transplants of anterior pituitary and succeeded in recovering fertilised blastocysts from the uterine horn, but none was implanted. The failure of implantation would seem to point to some inadequacy of endometrial preparation, but Engle, citing the experiments of Smith (1927) and Shelesnyak (1931), who produced microscopic deciduomata in immature rats after treatment with anterior pituitary implants, is of the opinion that some additional factor other than deficient maturation of endometrium is involved. He admits, however, that production of false deciduomata and a successful implantation are not wholly comparable. The absence of antimesometric hyperæmia in all of our treated immature guinea-pigs shows that the immature uterus does not respond "characteristically" to hormonal treatment, and it is therefore not surprising, but rather to be expected, that efforts to induce implantation in the immature animal have been universally unsuccessful.

The failure to achieve pregnancy in the immature animal by treatment with hormones finds an analogy in the relative sterility of puberty and adolescence. Hammond (1925) has shown that young rabbits will mate, ovulate, develop corpora lutea, and yet there is no pregnancy. The uterine condition is unfavourable for implantation. Mirskaya and Crew (1930 *b*) distinguish between puberty and sexual maturity which they define as "that stage of individual development characterised by the exhibition of the maximum fertility ratio and by the ability to produce viable offspring and to rear them." The authors have shown that the interval between the onset of puberty and the attainment of full sexual maturity in mice varies not only in different stocks, but also as between individuals of the same stock under different conditions. Hartman (1931) has noted an interval of a year between the first symptoms of puberty and the first conception in the rhesus monkey, and in his view "this staircase phenomenon of adolescence indicates a gradual though saltatory increase in the effect of the ovarian hormones." Maturity, according to him, is marked by the first ovulation and the preparation of a receptive uterus capable of carrying the offspring to term. This definition seems to suggest that a receptive uterus is contemporary with the first ovulation, which, however, is not confirmed by the experiments of Engle (1931) and Mirskaya and Crew (1930 *b*), who could produce ovulation in treated immature animals but not implantation. Hartman also has some interesting observations on the time-interval (2-3 years) between puberty and maturity in the human as observed in cases of early marriage. Reynolds (1939), reviewing the whole problem of fertility in

relation to sexual maturity, is of the opinion that the uterine tissue has to accustom itself to the periodic hormonal activity and that there is a period of "experience" following the first œstrus during which uterine tissue acquires its optimum capacity for pregnancy. The results of this apprenticeship should, however, be capable of expression in either quantitative or qualitative terms. Again, if one accepts with Mirskaya and Crew (1930 *a*) that the reason for the failure to procure pregnancy in treated immature animals is the lack of some "essential contribution from the immature soma," it ought to be possible to detect some direct effect on the genital organs of the lack of this essential contribution. If it be admitted that the failure to induce pregnancy in treated immature animals and the relative sterility of adolescence are but different aspects of the same fundamental problem, then in the light of the present experiments it must be accepted that the deficiency factor is not a mere defect in the hormonal balance but rather, as exemplified by the absence of typical vascular response, an inherent incapacity of the immature or pubertal uterus to react maturely to the hormonal stimulus. It is not considered that the absence of antimesometric hyperæmia in the uterus is the one obstacle to successful implantation in the treated immature or the pubertal female, but the observation offers so far the only concrete evidence of the inability of the immature uterus to respond maturely to hormonal stimulation.

SUMMARY AND CONCLUSIONS.

1. Twelve sexually immature guinea-pigs were treated with œstrin and progesterone. The animals were killed and the uteri sectioned, stained, and examined. Three untreated immature animals were used as controls.
2. With one exception the uteri of the treated animals were much enlarged and displayed other heat changes such as endometrial proliferation, active glands, etc.
3. Vaginal smears were taken frequently during the experiment. None of the animals exhibited typical œstrous smears.
4. The vascular response in most cases consisted of an increased but even distribution of blood-vessels. Occasionally there was a haphazard attempt to produce localised hyperæmia, which, however, was confined to a few sections of the sexually active portion of the uterus and was frequently misplaced.
5. None of the animals developed the characteristic antimesometric hyperæmia of the normal adult œstrous uterus.

6. The absence of this antimesometric hyperæmia denotes the inability of the immature uterus to respond maturely to hormonal stimulation.

7. These results offer eloquent argument in favour of the view that the failure to induce implantation in the immature animal by hormonal treatment is due to an inadequate preparation of the uterus.

ACKNOWLEDGMENTS.

We wish to acknowledge the helpful supervision of the Chief of the Department, Professor D. M. Blair, and a grant from the Rankin Research Fund in aid of the expenses of research. The hormones, Progynon B oleosum forte and Proluton, were kindly supplied by Messrs Schering, Ltd.

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DESCRIPTION OF PLATES.

All illustrations are untouched microphotographs of cross-sections of the sexually active (middle) portion of the uteri of immature guinea-pigs stained with the benzidine reaction.

All photographs were taken at the magnification of $\times 18$.

In every case the lower part of the picture represents the antimesometric, and the upper the mesometric, portion of the uterus.

PLATE I.

Fig. 1. Animal No. 2. Untreated control. Note small size of uterus and uniform distribution of blood-vessels.

Fig. 2. Animal No. 6. A "typical" heat response of the immature uterus to œstrin and progesterone administration. Well-marked endometrial proliferation. Generalised vascularity.

Fig. 3. Animal No. 9. Good attempt to produce antimesometric hyperæmia (confined to only a small portion of the sexually active part of the uterus).

Fig. 4. Animal No. 10. Subepithelial hæmorrhages simulating antimesometric hyperæmia.

PLATE II.

Fig. 5. Animal No. 5. Hyperæmia involving side walls of antimesometric portion, but leaving free the antimesometric pole.

Fig. 6. Animal No. 8. Well-developed but misplaced hyperæmia on side wall of uterus.

Fig. 7. Animal No. 14. Good endometrial and vascular reaction on one side of the uterus, while the other side displays no histological or vascular response whatsoever.



FIG. 1.

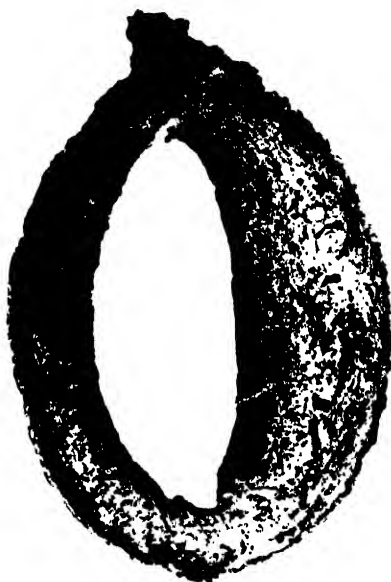


FIG. 2.



FIG. 3.



FIG. 4.

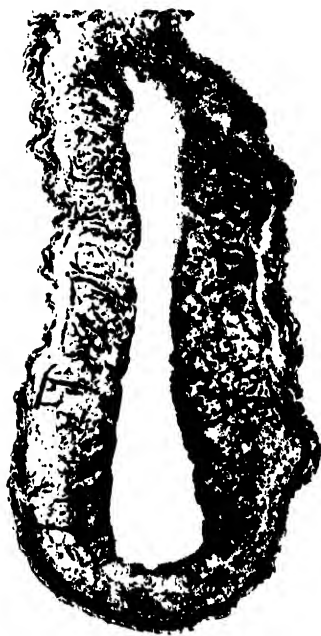


FIG. 5.

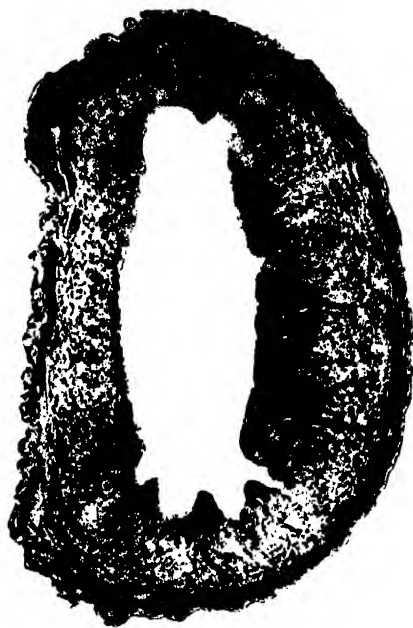


FIG. 6.



FIG. 7.

XVII.—A Study of the Cytoplasmic Inclusions during the Spermatogenesis of the Mouse. By **R. A. R. Gresson**, D.Sc., Department of Zoology, University of Edinburgh. *Communicated by Professor J. RITCHIE*, M.A., D.Sc. (With Two Plates.)

(MS. received January 16, 1942. Read March 2, 1942.)

CONTENTS.

	PAGE
I. INTRODUCTION	197
II. MATERIAL AND METHODS	198
III. OBSERVATIONS	198
1. The Spermatogonia	198
2. The Spermatocytes	198
3. Spermateliosis	200
IV. DISCUSSION	202
V. SUMMARY	206
VI. REFERENCES TO LITERATURE	207
VII. DESCRIPTION OF PLATES	208

I. INTRODUCTION.

IN previous contributions the writer has described the structure and behaviour of the mitochondria and Golgi material of the egg of the mouse (Gresson, 1933, 1938 and 1940 *b*), and more recently has traced the cytoplasmic inclusions through the stages of maturation, fertilisation and the first cleavage division (Gresson, 1941). As the result of observations on the sperm middle-piece it is claimed that the mitochondria of the sheath are liberated into the ooplasm of the fertilised egg, and are shared out between the first two blastomeres; and further, that a Golgi element is present in the middle-piece, and that this breaks up and its products are distributed through the cytoplasm of the fertilised ovum (Gresson, 1940 *a*, and 1941).

As observations were carried out on the cytoplasmic bodies of the ripe sperm only, it was considered desirable to trace the history of the mitochondria and Golgi material through the stages of spermatogenesis of the mouse. Consequently, the present work was undertaken primarily to determine the part played by the Golgi material and mitochondria in the formation of the sperm middle-piece.

Although a large amount of work has been recorded on the spermatogenesis of mammals, very little has been carried out with the aid of techniques for the demonstration of the Golgi substance. The results of such investigations as have been made on the cytoplasmic inclusions of the male germ-cells of mammals will be discussed later in this paper, in so far as they have a bearing on the present findings. Other works on spermatogenesis are mentioned in the papers cited.

II. MATERIAL AND METHODS.

The material used in the present investigation consisted of the testes of the mouse (*Mus musculus*). The testes were dissected out and small pieces immediately placed in the fixing fluid. Sections were subsequently cut at $5\ \mu$ and at $8\ \mu$ in thickness.

For the study of the mitochondria, material fixed in Flemming (without acetic), and treated according to the method of Champy-Kull, was found to be satisfactory. For the demonstration of the Golgi substance, material was prepared according to the methods of Mann-Kopsch, Kolatchev and Aoyama. The Kolatchev and Aoyama preparations gave the best results. The mitochondria were also visible in Aoyama sections, and the Golgi bodies, particularly those of the resting spermatocytes and of the young spermatids, were shown in some of the Champy-Kull preparations. Material was fixed in formol and the sections subsequently stained in Sudan IV according to the method of Kay and Whitehead (1935) for the demonstration of fat.

I wish to express my thanks to Professor James Ritchie for the interest he has shown in the work and for reading the manuscript.

III. OBSERVATIONS.

1. *The Spermatogonia.*

The mitochondria of the spermatogonia are large and spherical. They are most numerous in the neighbourhood of the nucleus, and are practically absent from the extreme periphery of the cell. The Golgi material is in the form of a flattened mass in close contact with one pole of the nucleus. It is probable that it is made up of individual elements closely packed together (Pl. I, fig. 6). Spermatogonia in division were not observed.

2. *The Spermatocytes.*

The mitochondria of the resting primary spermatocytes are spherical. They are situated in small clumps more or less closely surrounding the

nucleus; a few, however, may be scattered singly in the cytoplasm (Pl. I, fig. 1). Prior to division they become distributed through the cell (Pl. I, fig. 2), and during the prophase appear to be smaller and more numerous than in the resting stage (Pl. I, fig. 11). They remain scattered through the cytoplasm during the subsequent phases of nuclear division, but in the metaphase show a slight tendency to form small clumps in the vicinity of the spindle (Pl. I, figs. 3, 4, and 5). With the division of the cell they are distributed in approximately equal numbers to the second spermatocytes.

The Golgi material is in the localised condition, and is greater in amount than in the spermatogonia. It is well shown in Aoyama and in Kolatchev preparations and is present in some of the Champy-Kull sections. When it is not visible in Champy-Kull material the idiosome or archoplasm is frequently shown as an area which stains more deeply than the surrounding cytoplasm. In some of the younger primary spermatocytes the Golgi material is situated some distance from the nucleus, but later it is in close contact with the nuclear membrane, when it often appears as a homogeneous, deeply impregnated mass (Pl. I, fig. 7). Examination of a large number of sections indicates that the Golgi mass is made up of individual elements, probably in the form of rods, threads, and granules, which closely invest the archoplasm (Pl. I, figs. 8, 9). In some cases ring-shaped elements are situated at the periphery of the Golgi mass (Pl. I, fig. 10).

In the prophase of the division of the primary spermatocytes the Golgi material forms a more compact deeply impregnated mass situated close to the nuclear membrane (Pl. I, fig. 11). In sections containing metaphase figures the Golgi material is shown as a clump of rods and granules lying close to the spindle in the equatorial region. The elements are more widely separated than in the resting condition or during the prophase (Pl. I, figs. 12, 13). During the anaphase and early telophase the Golgi material is still situated in the equatorial region (Pl. I, fig. 14). Cells in the late telophase were not identified, but there is little doubt that the Golgi substance, owing to its equatorial position during the phases of division, is divided passively into two with the division of the cytoplasm. It should be pointed out that of the Golgi preparations, division figures were shown clearly in the Aoyama material only. Consequently, it was not possible to examine the Golgi material, during the stages of cell division, in the Kolatchev sections.

The behaviour of the mitochondria and Golgi material during the division of the second spermatocytes appears to be similar to that of the first division.

3. *Spermateleosis.*

Granular mitochondria are present in the young spermatids. They are, at first, smaller and less numerous than those of the spermatocytes, but later increase in size and number.

The Golgi material of the young spermatids surrounds the idiosome and is in contact with the anterior pole of the nucleus (Pl. I, fig. 15). At this stage, and during the formation and early growth of the acrosome, the Golgi material is visible in the Champy-Kull as well as in the Aoyama and Kolatchev preparations. Although the localised Golgi substance of the spermatid often appears to have the structure of a network, careful examination indicates that, in reality, it consists of rod-shaped elements which closely invest the archoplasm. Owing to the density of the Golgi material surrounding the idiosome it was not possible to identify pro-acrosomic granules or to follow the very early stages of the formation of the acrosome, nor was this possible in preparations in which the Golgi substance was not impregnated.

The Golgi material becomes somewhat flattened over the anterior pole of the nucleus, and, later, moves away from the nuclear membrane, revealing a clear unstained area or vacuole which is closely applied to the anterior pole of the nucleus so that the nuclear membrane is slightly depressed. In Champy-Kull preparations the clear area is seen to contain a large deeply stained granule in contact with the nuclear membrane (Pl. I, fig. 16). The granule is the developing acrosome. The vacuole and the granule increase in size so that the Golgi material is pushed farther away from the nucleus (Pl. II, fig. 17), becoming free and migrating towards the posterior part of the cell. At the same time the vacuole and the acrosome spread out over the anterior pole of the nucleus which regains, more or less, its original shape (Pl. II, fig. 18). The Golgi material, which is now in the vicinity of the posterior pole of the nucleus, is very compact and divides into a small and a larger part. The axial filament is now present and the proximal and distal centrioles can sometimes be identified in Champy-Kull sections (Pl. II, figs. 18, 19). After the Golgi material has reached the neighbourhood of the posterior pole of the nucleus, the cell begins to elongate, and the mitochondria increase in size and often appear as spherical bodies with a deeply stained cortex. In some sections a body which stains more deeply than the surrounding cytoplasm is shown in contact with the acrosome vacuole. This is part of the archoplasm (Pl. II, fig. 19). With the further elongation of the cell the small spherical piece of Golgi material separates from the main mass and moves towards the region of the centrioles,

becoming smaller and more compact as it does so. The residual Golgi material moves towards the posterior part of the cell and becomes less deeply impregnated. It surrounds a material which stains more deeply than the cytoplasm, and is part of the archoplasm. The mitochondria are spherical but stain homogeneously and are distributed through the cytoplasm (Pl. II, figs. 20, 21). While these changes have been taking place the acrosome continues its development, increasing in size at the expense of the outer clear area (Pl. II, fig. 20), which it soon fills completely. The Golgi body, which has previously separated from the main mass, is now in close proximity to the centrioles (Pl. II, fig. 21).

The nucleus now rapidly elongates and the head of the sperm assumes its characteristic form. At this stage the acrosome is lightly stained, and, with the technique used during the present investigation, is difficult to differentiate from the rest of the head region (Pl. II, figs. 22, 23).

After the differentiation of the head region, a deeply impregnated granule is visible lying in the region of the centrioles immediately posterior to the nucleus. This granule is present in Champy-Kull, Aoyama, and in Kolatchev preparations, and is identified as the Golgi substance which, at an earlier stage, is budded off from the main mass of Golgi material and migrates to the posterior pole of the nucleus (Pl. II, figs. 23, 24, 25, 26, 27). Its close proximity to the centrioles makes the certain identification of the latter very difficult. The residual Golgi material, as seen in Aoyama sections, still surrounds the archoplasm but appears to be breaking up into smaller pieces (Pl. II, fig. 27).

The majority of the mitochondria soon collect into large clumps situated in the vicinity of the axial filament. At this stage the residual Golgi material is not shown in the Champy-Kull sections. Vacuoles make their appearance in the cytoplasm (Pl. II, fig. 23).

After the mitochondria become clumped they move closer to the axial filament, and finally become arranged around the filament to form the mitochondrial sheath of the middle-piece of the sperm. A few mitochondria remain in small clumps and scattered singly in the residual cytoplasm. At first the mitochondria are arranged loosely around the axial filament (Pl. II, fig. 24), but later become more compact (Pl. II, fig. 25). The Golgi material is still visible in the neck region of the future sperm (Pl. II, fig. 25), but that of the residual cytoplasm has broken up and is becoming scattered. Examination of Kolatchev preparations of late spermatids shows that the residual cytoplasm contains a large number of deeply impregnated globules and granules, which are identified as fat. A small number of vacuoles are also present (Pl. II, fig. 26). That the globules and granules are of a fatty nature was

confirmed by an examination of sections prepared according to the method of Kay and Whitehead (1935) for the identification of fat. The spermatocytes and early spermatids do not contain any material which stains with Sudan IV, while brightly coloured globules and granules are present in the residual cytoplasm of the late spermatids.

The Golgi granule was identified in the neck region of very late spermatids, and in nearly ripe and ripe spermatozoa (Pl. II, fig. 28). Not only was this substance traced from its origin, from the Golgi material which had moved away from the growing acrosome, but its appearance and reactions in Champy-Kull, Aoyama, and Kolatchev sections leave little doubt that it is composed of Golgi substance.

Living spermatocytes and spermatids were examined in smear preparations mounted in a weak solution of neutral red, and also unstained. While the idiosome complex was visible in a number of cells, the Golgi material was so faintly shown that it is unsafe to draw definite conclusions regarding the form of the Golgi substance, beyond stating that it does not appear to be in the form of a network.

IV. DISCUSSION.

The most complete account of the cytoplasmic inclusions during all stages of the spermatogenesis of a mammal appears to be that contained in the papers of Gatenby and Woodger (1921) and Gatenby and Wigoder (1929) for the guinea-pig. The history of the cytoplasmic inclusions of the male germ-cells of the mouse is in general similar to that of the guinea-pig. There are, however, certain differences.

The mitochondria of the guinea-pig, according to Gatenby and Woodger, are scattered through the cytoplasm during all stages of the spermatocytes. According to Duesberg (1910), however, they are at first situated in relation to the idiosome, and later form small groups around the nucleus. Finally, they become scattered through the cell. In the young primary spermatocytes of the mouse the mitochondria are situated in the vicinity of the nucleus, becoming dispersed prior to the prophase of the first division. During division the behaviour of the mitochondria is closely similar to that of the guinea-pig as described by Gatenby and Woodger. In the late spermatid of the guinea-pig the mitochondria do not form large clumps as in the mouse. The majority finally become arranged around the axial filament, where they are figured by Gatenby and Woodger as large discrete granules. Those which remain in the residual cytoplasm run together and undergo degenerative changes. In both the mouse and the guinea-pig the majority of the

mitochondria take part in the formation of the middle-piece of the sperm, while a few are eliminated with the residual cytoplasm.

Sjövall (1906) describes and figures a structure which blackens with osmic acid and is situated in relation to the idiosome of the spermatogonia and spermatocytes of the mouse. He states that it is identical with the "Golgi-Kopsch network" of somatic cells. In the spermatid this structure leaves the nucleus and passes to the posterior part of the cell. Sjövall does not, however, give a detailed account of the behaviour of the Golgi material nor of the formation of the acrosome.

The origin of the acrosome of the rat was traced by Duesberg (1908). Clear areas make their appearance in the idiosome. Later, a granule arises in each area, and these granules run together to form a single granule surrounded by a large vacuole-like structure. The subsequent stages in the history of the vacuole and granule appear to be similar to those of the mouse as described in the present paper. Duesberg describes a deeply stained body which he calls the "corps chromatode." In the young spermatid it is situated in the neighbourhood of the idiosome. Later, it passes to the vicinity of the centrioles and renders their observation difficult. It is, at first, irregular in shape, but becomes spherical, breaks up into two or three pieces which finally disappear in the cytoplasm after the elongation of the cell. Although Duesberg's figures are from Flemming preparations, and the "corps chromatode" is not shown surrounding the idiosome of the young spermatid, there is little doubt that this structure is the Golgi material. He does not trace part of the material on to the middle-piece of the sperm.

The Golgi material of the spermatocyte and of the spermatid, according to Gatenby and Woodger, is in the form of curved plates and rods surrounding the archoplasm. In silver preparations it appears as either a reticulum or as a homogeneous body. That the localised Golgi material is made up of discrete bodies was confirmed by Subba Rau and Brambell (1925), who carried out observations on the living spermatocytes and early spermatids of the guinea-pig. The present writer concludes that the Golgi material of the developing male germ-cells of the mouse is in the form of rods and granules. The Golgi elements are, however, small and closely packed together around the archoplasm, making observation of their shape extremely difficult. In the guinea-pig the Golgi bodies are fewer in number than in the mouse, and Gatenby and Woodger were able to trace the proacrosomic granules from the spermatocyte until they ran together to form the proacrosome of the spermatid.

The number of mammals in which spermatogenesis has been investigated with methods for the demonstration of the Golgi material is

small. The conditions in the animals observed indicate that there is wide variation in the behaviour of the Golgi substance during the division of the spermatocytes. According to Gatenby and Beams (1935) the localised Golgi material of the human spermatocyte breaks up, during the prophase, into small granules. In the later stages of division the granules tend to lie near the spindle, but not necessarily near the asters. During the telophase they are situated close to the nucleus on the side away from the asters. In the young spermatid the Golgi granules are somewhat scattered to one side of the cell. Later, they come together to form a group situated close to the nucleus.

The location of the Golgi material during the stages of division of the spermatocytes of the mouse is in marked contrast to that of the rat as described by Ludford and Gatenby (1921), and to that of the guinea-pig as described by Gatenby and Woodger. In the guinea-pig approximately half of the Golgi elements are grouped round each pole of the spindle. In the rat the Golgi bodies are scattered through the cell during the metaphase, and in the telophase form two groups situated towards opposite ends of the cell. The conditions in the mouse more closely resembles that of the opossum as recorded by Duesberg (1920). In the opossum the Golgi material does not break up into granules until the anaphase, when it forms one or more clumps situated between the chromosomes. The present writer observed, in silver preparations, a large number of spermatocytes undergoing prophase changes. The Golgi material was shown clearly as a clump of elements lying close to the nuclear membrane. Cells in the metaphase, anaphase, and telophase were not numerous, but in some of these the Golgi material was visible in the equatorial region. In no case were scattered Golgi elements observed near the spindle poles. It is concluded, therefore, that in the mouse the Golgi material remains in a single clump and is separated passively into two smaller groups with the division of the cell, or that, as in the opossum, it does not break up into granules until immediately before cell division. Examination of a large number of young spermatids appears to add further support to the view that the Golgi material remains in a single clump. Cells were never seen in which the Golgi elements were coming together from the dispersed condition, as might be expected if the elements were distributed through the cytoplasm.

The acrosome of the mouse is first visible after the Golgi material begins to move away from the nucleus: there is little doubt that the acrosome is formed under the influence of the Golgi material. It is probable that it arises from proacrosomic granules which run together in a similar manner to those of the guinea-pig.

Gatenby and Woodger state that the proacrosome of the guinea-pig is surrounded by a vacuole, and that it becomes divided into an inner and an outer zone. In the mouse the clear area which, at first, surrounds the developing acrosome corresponds with the vacuole of the guinea-pig. An inner and an outer zone were not identified. According to Gatenby and Beams (1935) the human acrosome is not divided into an inner and an outer zone. The formation of the acrosome of the mouse is closely similar to that of the dog as described by Bell (1929), and also shows resemblance to that of the human spermatid. In the spermatid of the dog the Golgi material is in the form of granules which surround the idiosome. A large vacuole is formed between the idiosome and the nucleus. With the increase in size of the vacuole pressure is exerted on the anterior pole of the nucleus, and a granule, which gives rise to the acrosome, is formed. The subsequent changes undergone by the vacuole and granule are closely similar to those of the corresponding structures of the mouse.

Gatenby and Woodger state that, at the stage when the spermatid of the guinea-pig is elongating, a small part of the Golgi material and archoplasm becomes detached from the main mass and migrates to the protoplasmic bead of the middle-piece. The present findings on the origin of the Golgi material of the sperm confirm those of Gatenby and Woodger. Subba Rau and Brambell (1925) believe that the Golgi material of the middle-piece is, in some cases at least, budded off from the main mass at an earlier stage than that described by Gatenby and Woodger, and may become separated before the Golgi substance has moved away from the acrosome. During the present investigation it was found that the time at which the Golgi material of the middle-piece is budded off varies slightly. Budding was not observed while the Golgi substance was in contact with the acrosome. In most cases it occurs as the Golgi material migrates towards the posterior pole of the nucleus, but sometimes does not take place until it has reached the posterior part of the cell.

Subba Rau and Brambell observed Golgi material in living spermatocytes and spermatids of the mouse. According to their figures the Golgi elements are larger and fewer in number than those shown in the fixed material used during the present investigation. They do not describe the Golgi substance of the late spermatid nor that of the sperm. The observations of the present writer on living material added little to the findings based on the examination of fixed and stained preparations.

Gatenby and Woodger figure the Golgi material of the sperm middle-piece of the guinea-pig as consisting of rod-shaped elements. Later,

Gatenby and Wigoder (1929) give figures of ripe sperms in which the Golgi material appears to be in the form of a network. They do not, however, describe its structure. In the mouse the Golgi substance of the middle-piece originates from a small rounded body, which in some of the sections appears to be in the form of a deeply impregnated cortex surrounding a small amount of archoplasm. After it has taken up its position in the middle-piece it is visible as a deeply impregnated granule.

The present investigation shows that part of the original Golgi material of the spermatid is present in the middle-piece of the ripe sperm of the mouse. Golgi granules were noted by Bell in the middle-piece of the sperm of the dog. Gatenby and Woodger, and Gatenby and Wigoder have described Golgi material in the spermatozoa of the guinea-pig. Gatenby and Beams do not record the presence of Golgi material in the middle-piece of the human sperm. It is evident that Golgi material is present in the middle-piece of the majority of those mammals examined with the aid of modern osmic and silver techniques.

Bell (1929) states that the Golgi granules of the spermatid of the dog become aggregated to form large spheres in the residual cytoplasm. He believes that the Golgi material is probably an unsaturated lipoid and that the large spheres to which it gives rise are neutral fat. The present investigation shows clearly that the Golgi material in the residual cytoplasm of the mouse undergoes degenerative changes and is converted into fat. According to Gatenby and Beams the Golgi remnant of the human spermatid does not give rise to fat.

The post-nuclear granules and body described by Gatenby and Wigoder for the spermatid of the guinea-pig, or the post-nuclear cap recorded by Gatenby and Beams for the human sperm, were not observed during the spermatelcosis of the mouse.

V. SUMMARY.

1. Spherical mitochondria are present in the spermatogonia and spermatocytes. In the spermatogonia they are most numerous in the neighbourhood of the nucleus. In the resting primary spermatocytes they are, for the most part, in small clumps surrounding the nucleus. Prior to division they spread out through the cytoplasm and remain scattered through the cell during the phases of division of the first and second spermatocytes.

2. The Golgi material of the spermatogonia and of the spermatocytes is situated at one pole of the nucleus. It consists of individual elements surrounding the idiosome. During nuclear division it remains in the

localised condition, in the equatorial region, and is either divided into two smaller groups by the division of the cell, or disperses through the cytoplasm immediately before cell division.

3. The mitochondria of the young spermatid are granular. Later they increase in size, become clumped, and, finally, the majority surround the axial filament.

4. The Golgi material of the young spermatid is closely applied to the anterior pole of the nucleus. It moves away, revealing a large vacuole which contains a deeply stained granule. The granule increases in size, at the expense of the vacuole, and gives rise to the acrosome.

5. The Golgi material moves away from the nucleus. It buds off a small spherical piece of Golgi substance which migrates to the posterior pole of the nucleus and, later, is included in the middle-piece of the sperm.

6. The Golgi remnant breaks up in the residual cytoplasm and is converted into fat.

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VII. DESCRIPTION OF PLATES.

LETTERING.

Ac., acrosome; A.F., axial filament; Ar., archoplasm; C., centrioles; Ch., chromosomes; C.V., vacuole in residual cytoplasm; F., fat; G.B., Golgi bud; G.M., Golgi material; G.R., Golgi remnant; M., mitochondria; M.S., mitochondrial sheath; N., nucleus; S.H., sperm head; S.G.M., sperm Golgi material; V., vacuole surrounding developing acrosome.

PLATE I.

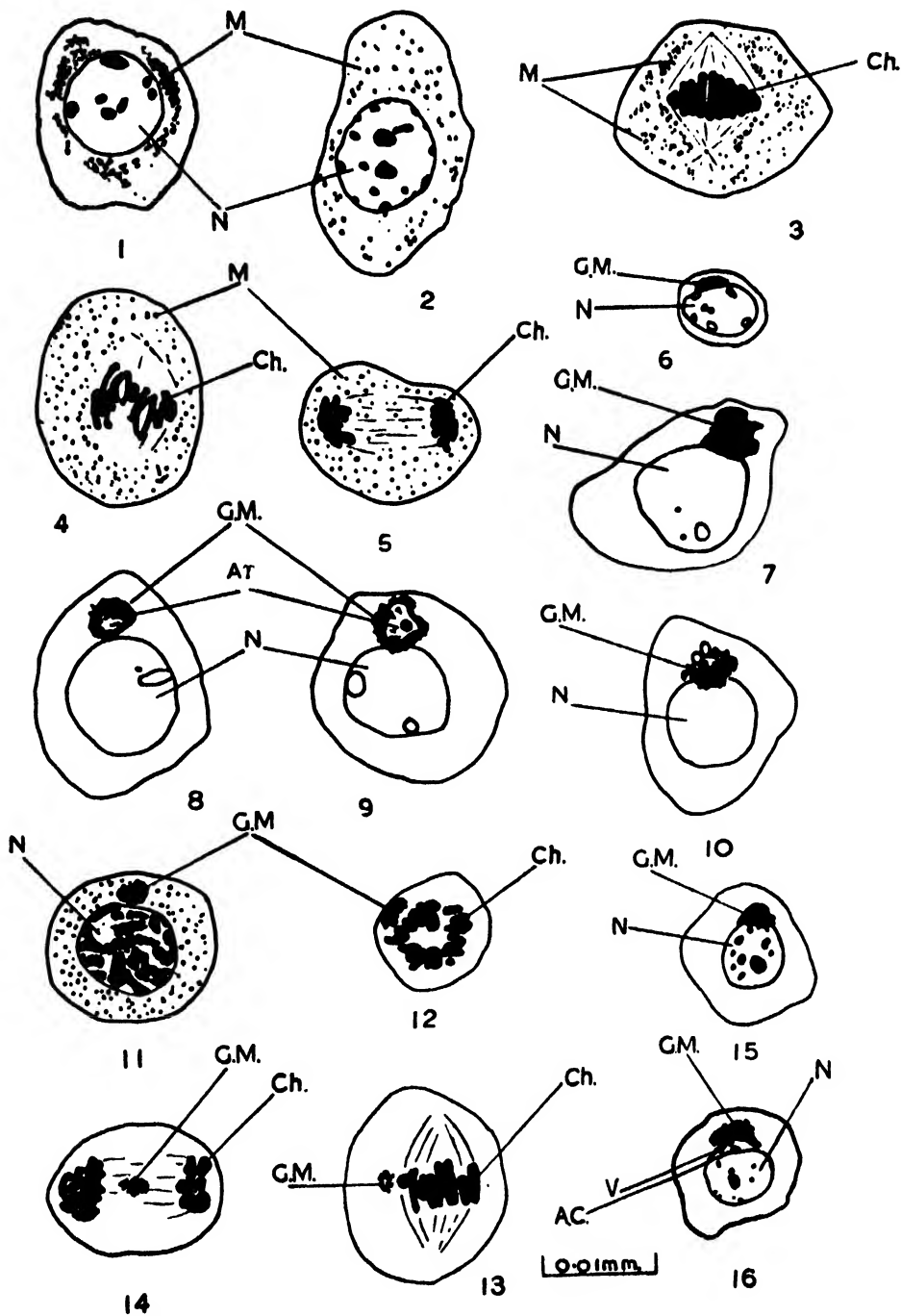
Figs. 1-5 from Champy-Kull preparations. Figs. 6-10 and 15 and 16 from Kolatchev preparations. Figs. 11-14 from Aoyama preparations.

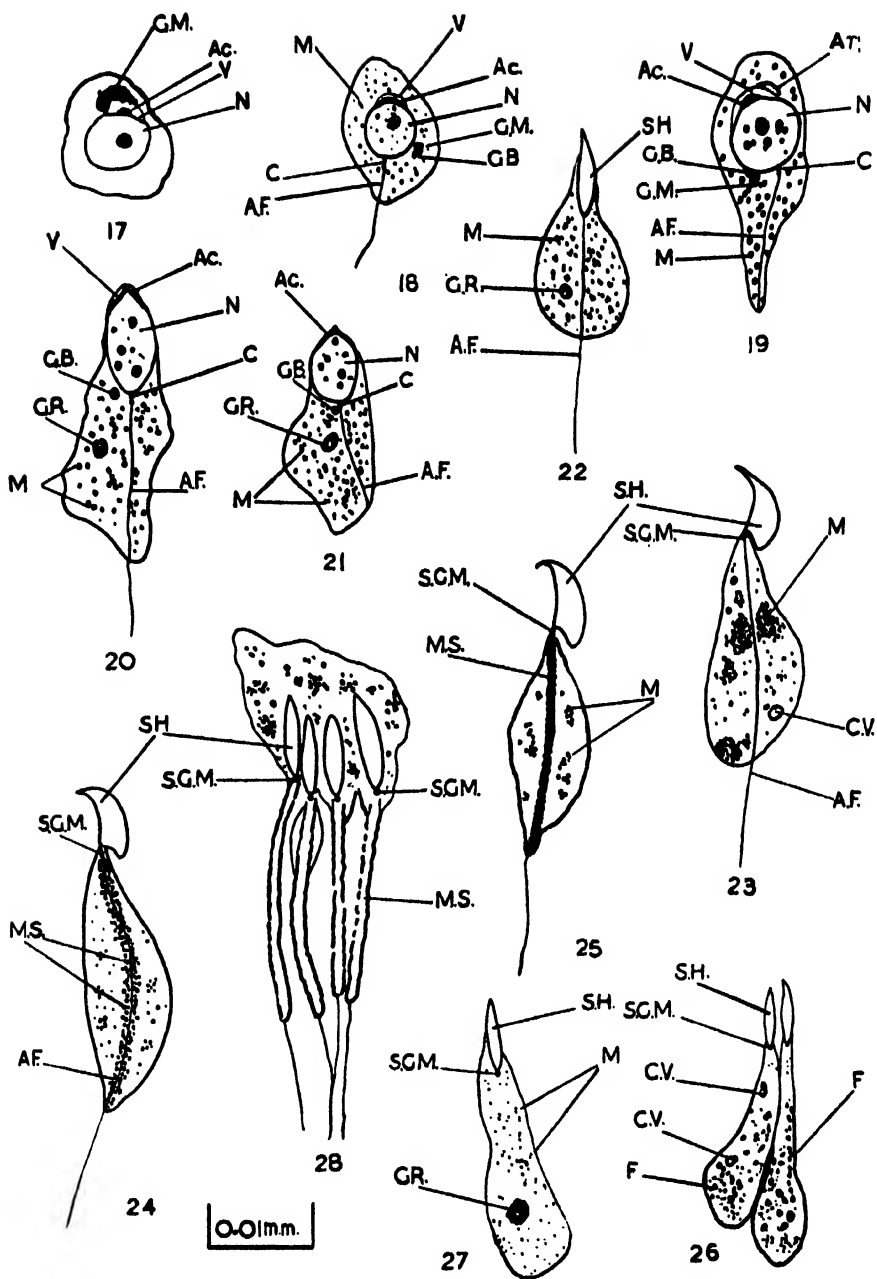
Figs. 1, resting primary spermatocyte; 2, first spermatocyte just before division; 3, first spermatocyte: metaphase; 4, first spermatocyte: early anaphase; 5, first spermatocyte: late anaphase; 6, spermatogonium; 7-10, spermatocytes to show Golgi material; 11, first spermatocyte: early prophase; 12 and 13, spermatocyte: metaphase; 14, spermatocyte: early telophase; 15 and 16, young spermatids.

PLATE II.

Figs. 17, 26, and 28 from Kolatchev preparations. Fig. 27 from Aoyama preparation. All other figures from Champy-Kull preparations.

Figs. 17, young spermatid; 18-25, spermatids showing stages in development of acrosome and history of mitochondria and Golgi material; 26, spermatids to show fat in residual cytoplasm; 27, spermatid to show Golgi remnant; 28, nearly ripe sperms to show Golgi material in the middle-piece.





XVIII.—Variation in *Gryphæa incurva* (Sow.) from the Lower Lias of Loch Aline, Argyll. By Rhona M. MacIennan, B.Sc., and A. E. Trueman, D.Sc. (With Eleven Text-figures.)

(MS. received January 27, 1942. Read March 2, 1942.)

INTRODUCTION.

VARIOUS species of the lamellibranch *Gryphæa* have received much attention from palæontologists in the past twenty years, partly because careful collecting appears to have produced continuous series of forms (lineages) and partly owing to the problems which are raised by their progressive development from more normal oysters.

The series leading to *Gryphæa incurva* (Sow.) has received most attention; its range and variation were summarised in 1922 (Trueman), although references to its evolution had been published earlier (Jones, 1862; Kitchin, 1912). The significance of the range of variation within the lineage and its plexus-like nature were discussed later (Trueman, 1924, 1929, 1930). Subsequently Professor H. H. Swinnerton has devoted much attention to variation in members of this series, first in 1932 and more recently in two Presidential Addresses to the Geological Society (1939, 1940). The series has been held to provide a clear example of palingenesis, the development of the individual shell presenting stages closely comparable with those of its ancestors (George, 1933; Swinnerton, 1938). Other writers have concerned themselves with the apparent parallelism shown in the evolution of later series of *Gryphæa* (Lang, 1923; Arkell, 1933; Trueman, 1940).

In short, *Gryphæa* has become one of the most discussed genera of invertebrate fossils, and in the analysis of its lines of descent evidence appears to have been gathered which throws light on the more general problems of evolutionary mechanics and of the nature of species. For these reasons, the genus has been quoted in many recent biological works (e.g. De Beer, 1940; Robson, 1928; Waddington, 1939).

The specimens here described are late members of the *G. incurva* series, collected from the Lower Lias (Bucklandi Zone) of Loch Aline, Morvern, Argyllshire (Lee and Bailey, 1925, p. 77). In the works referred to, all the shells whose variation has been discussed have been

collected in England (at places southwards from Lincolnshire) and in South Wales. The present group thus affords comparative material from a rather distant habitat, situated near the shore line of the northern arm of the British Liassic sea. In the course of this study attention has been given to the curvature of the shell (which has been neglected in recent work) and to the possible relations of the developing *Gryphæa* to its environment. In order to gain some information concerning the growth of oysters, one of the writers (A. E. T.) paid a visit to the oyster breeding station at Conway, where he received much assistance from the staff, especially Messrs R. E. Savage and H. A. Cole.

The thanks of the writers are also extended to Dr L. R. Cox for information concerning the genotype of *Gryphæa*, to Professor C. M. Yonge for references to the literature of modern oysters, and to Dr E. Neaverson, Dr J. Pringle and Dr Henry Woods for helpful discussion.

MATERIAL AND METHODS.

Approximately 400 specimens of *Gryphæa incurva* were collected at a horizon near the top of the Broadford Beds, at a point on the western shore of Loch Aline, just south of an old jetty (Lee and Bailey, 1925, p. 77). Great numbers of shells are available at that horizon, most of them large and presumably adult: few other shells occur apart from occasional ammonite fragments (*Arnioceras* sp. and cf. *Coroniceras* sp.) and in some shale layers, *Oxytoma inaequivalve* (Phill.). The limestones and shales below this horizon are rich in *Gryphæas*, but they are scarce or absent from the succeeding shales; 20 feet above the *Gryphæa*-beds, *Asteroceras* and *Microderoceras* have been collected.

The specimens were cleaned and a considerable number were dissected to show details of the umbonal region: of these about 70 were sufficiently complete to give accurate measurements, and the variation statistics given refer to this group. There is every reason to believe that it is typical of the community present at that level at Loch Aline.

The specimens form an apparently homogeneous group but show marked variation in many features. They agree with the *Gryphæa* communities previously described from the upper part of the Bucklandi Zone in having a curvature representing upwards of three-quarters of a whorl (Trueman, 1922, p. 263). As remarked by Lee (Lee and Bailey, p. 77), they are often markedly sulcate; this is especially notable during the middle stages of growth.

Measurements were made of the total length of the shell (referred to later as L), of the length of the right (opercular) valve (l), of the height

at right angles to L (H), of the height of the umbonal portion above the right valve (h) and of the width of the shell (W). The length of the area of attachment was measured, and the angle between it and the outer curve of the contiguous shell was determined approximately. The total length of the curve of the shell, measured along the convexity of the left valve, is referred to as CL. These various dimensions are indicated on the diagram, fig. 1.

Attempts were made to determine accurately the spiral angle, on the assumption that the curvature of a *Gryphæa* corresponds to a logarithmic spiral (D'Arcy Thompson, 1917, p. 577; Trueman, 1922, p. 261). The shadows of the specimens were projected on a screen, and their enlarged outline was accurately drawn on paper and cut out.* These curves

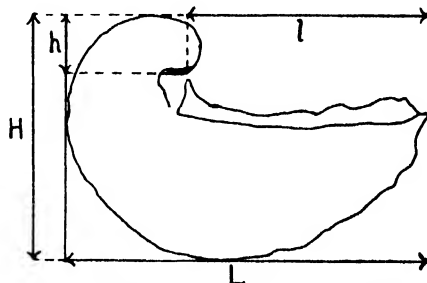


FIG. 1.—Diagram to show the measurements taken on shell of *Gryphæa*. L, total length of shell; l , length of right valve; H, total height at right angle to L; h , height of umbonal part of shell.

were then fitted against a series of logarithmic spirals drawn for spiral angles at intervals of one degree from 60 degrees to 82 degrees.

Some of the measurements noted above corresponded with those made by Swinnerton in his study of *Gryphæas* from other areas, and comparisons with his results are discussed below. His published graphs of adult *Gryphæas* are rather inadequate for a full discussion of communities from different areas, and some additional measurements have been made in order to compare the shape of the young stages with those in his communities. In his most recent address (1940) he studied features referred to as the "umbonal profile" and the "shell outline" of the juvenile community: for these measurements he selected (in each adult *Gryphæa*) "a stage of growth comparable in size with the adult *Ostrea irregularis* Münst. This naturally varied from specimen to specimen." As the resulting graphs do not show the actual dimensions, but a series of percentages, no indication of the sizes measured can be gained from them. Professor Swinnerton kindly informs the writers that his method was to select a prominent growth line marking a young stage on an adult shell; this involved some variation in the actual size of the young shell measured, and the young shells of *Gryphæa* so measured were on the whole shorter than the *Ostrea irregularis*. The choice of length was partly influenced by stage attained—that is, by similarity to *Ostrea irregularis*. For the chosen growth line the dimensions corresponding to

* The authors are indebted to Dr A. T. J. Dollar for suggestions as to this procedure.

length, breadth, and height were measured: the ratio of height-width was named the umbonal profile, the width-length ratio was called the outline. It is apparent that, notwithstanding the care taken in measuring these dimensions, the ratios (being determined on varying sizes) are somewhat unsatisfactory for statistical purposes, but for the sake of comparison, ratios as nearly as possible comparable have been determined for the Loch Aline community.

THE CURVATURE OF THE SHELL AND SHELL STRUCTURE.

The profile of the left valve of *Gryphæa incurva* forms approximately a logarithmic spiral. In many shells, however, there are obvious irregularities in growth; these are especially frequent at an early stage, where there is often a sharp turn in the profile (compare Swinnerton, 1939, fig. 3 *b*). In general, however, the profile of the valve shows a reasonably uniform curvature.

It has already been pointed out (Trueman, 1922, p. 261) that early members of the *Gryphæa incurva* series form a spiral with a low spiral angle, succeeding members of the series showing a gradual increase in the spiral angle leading to the more incurved *Gryphæas*. It has also been shown that in the development of the shell of these later *Gryphæas* a somewhat similar change from a lower to a higher spiral angle can be traced. Study of the Loch Aline *Gryphæas* confirms in detail these conclusions; the early stage of these shells frequently shows a spiral angle comparable with that of the adult of *Gryphæa obliquata* (Sow.). As regards this character, therefore, it may be said that the development is truly palingenetic (contrast Waddington, 1939, p. 242).

When profiles of the left valves are placed against accurate logarithmic spirals it is rare to find cases in which the curves fit closely for any considerable distance (fig. 2). Frequently, however, the early part of the shell (that is to a length of about 30 mm.) has a curve agreeing with a spiral angle of from 60 to 70 degrees. The main part of the shell, from a length of about 30 mm. to 50 mm., more often has a curve corresponding with a uniform spiral angle and this most frequently is near 71 to 73 degrees; the range of spiral angles observed at this stage of growth in the community is from 70 degrees to 77 degrees.

The amount of change in the spiral angle during the growth of an individual is very variable. In some cases a shell with an early angle of 60 degrees reaches 71 degrees in the adult stage, while another specimen changes from 62 to 73 degrees. A change from about 66 to 70 or 74 degrees is more frequent, while occasional specimens commencing with

an angle of 74 degrees proceed to 77 degrees. In a few cases little change in the spiral angle can be observed, and these appear to be individuals in which the early angle is high (fig. 2, *b*); thus one specimen has an angle of about 73 degrees throughout.

Since the spiral angle appears to be changing steadily in most examples in the Loch Aline community, a close fit to the calculated curves cannot

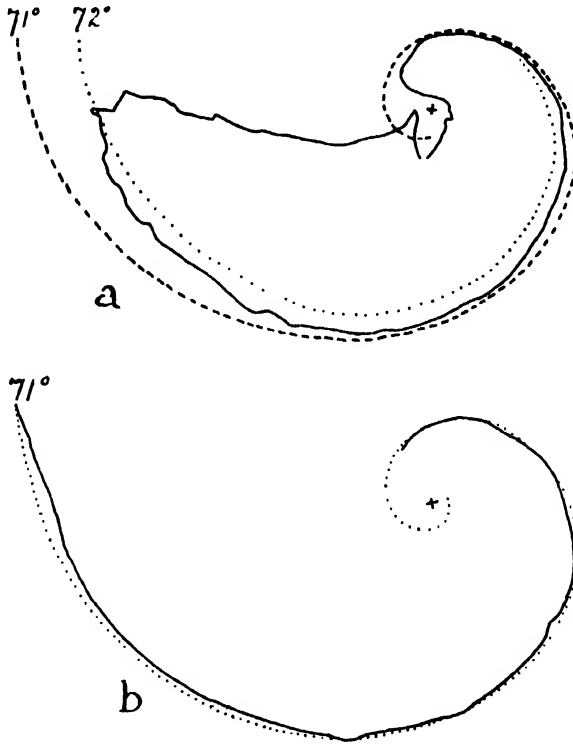


FIG. 2.—Diagrams showing relation of curvature of selected Loch Aline shells to particular logarithmic spirals. *a*, with spiral angle showing marked increase in later part, Hunterian Museum, No. S. 7741; *b*, showing fairly uniform spiral angle, Hunterian Museum, No. S. 7740.

be expected. In some cases, however, it is impossible to fit any portion of the later part of the shell to a curve unless the position of the origin is changed from that of the first part. In all such cases movement was apparently for a distance of several millimetres away from the umbonal part of the shell.

In many specimens there is a more distinct change in curvature in the last stages of growth (that is for about the last quarter of the whorl). In a fair proportion of the shells this corresponds to a rather sudden

increase in the spiral angle to 76 or 80 degrees, giving a much more incurved form of shell. Occasionally, however, the shell at this last stage shows a tendency to a reverse change in its coiling and a few specimens show a distinct "flare" along the ventral border (see fig. 3 and compare Trueman, 1922, fig. 6, p. 264).

The shell structure corresponding with this curvature has been studied both in sections of the shells and in "cellulose pulls." A diagram of

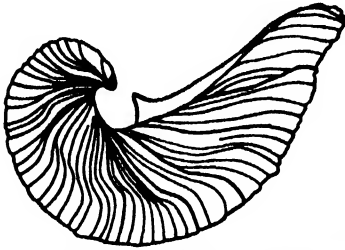


FIG. 3.—Large specimen of *Gryphæa incurva* from Loch Aline, showing tendency to form "flare" in later growth associated with movement of the organism from the left umbo. Note late flattening of growth lines (accompanying loss of sulcus). Slightly reduced.

Hunterian Museum, No. S. 7742.



FIG. 4.—Section of *G. incurva* from Loch Aline, drawn from a cellulose pull. Slightly reduced. Hunterian Museum, No. S. 7741.

such a section is shown in fig. 4. It will be noticed that the outer layer of the left valve of the shell is approximately 2 mm. thick and is made up of lamellæ inclined at a small angle to the curve of the shell. The main mass of the shell, however, consists of the enormously thick inner deposits; these very obviously were deposited by the inner surface of the mantle away from the growing edge of the shell. It is clear also that during growth the body cavity moved away from the position under the umbo of the left valve. To some extent the amount of this movement is reflected in the width of the cardinal area. This is marked by lines which represent the outcrops of the successive inner deposits.

From a consideration of the section it is also apparent that changes in the size of the cardinal area and in the degree of coiling must have affected the relative sizes of the left and right valves. In the more involute *Gryphæas* the right valve is small in relation to the whole shell, while in those forms with lower spiral angle, or those in which the angle is reduced in the last portion of the shell, the right valve is proportionately large. In this connection it may be remarked that the size of the right valve is approximately the same as the superficial area of the organism. It is clear that shells in the community show very considerable variation in the relative bulk of organism and its shell.

VARIATIONS IN SHELL FORM.

In all the features studied the specimens appear to represent a homogeneous community exhibiting wide variation in certain characters. The

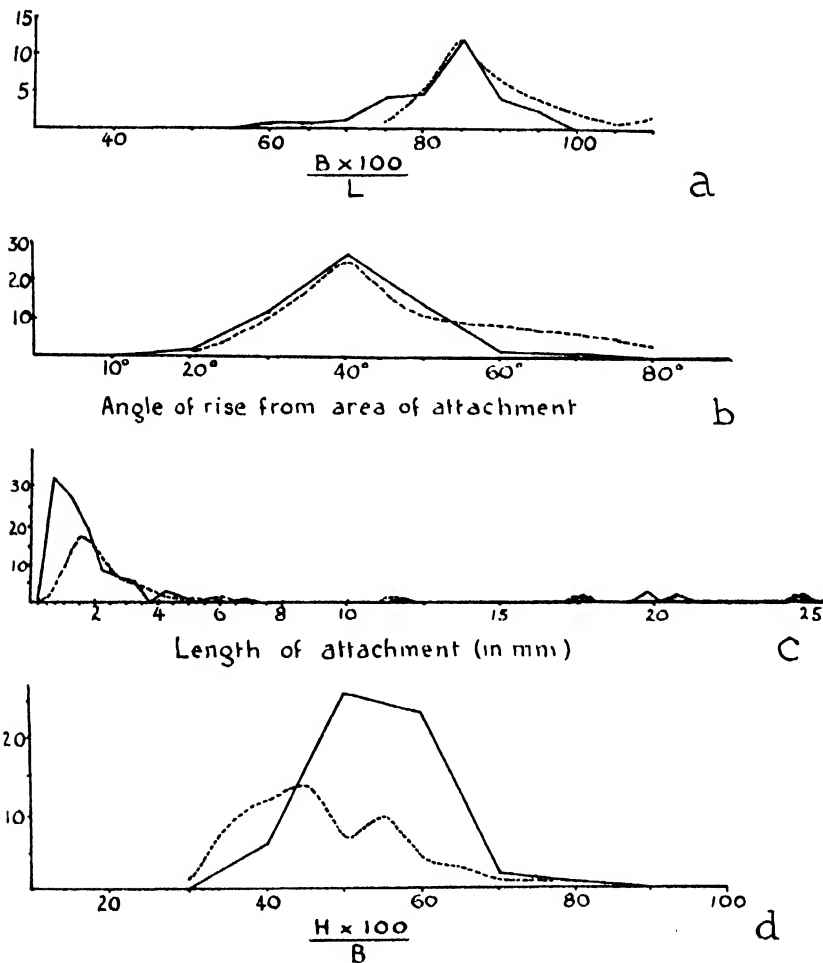


FIG. 5.—Graphs showing variations in certain features of the young growth-stages of *G. incurva* from Loch Aline: dotted lines show for comparison the variation in *G. incurva* from Fretherne, after Swinnerton (1939, fig. 5). *a*, breadth as percentage of length; *b*, angle of rise of profile from area of attachment; *c*, length of area of attachment; *d*, height as percentage of breadth.

specimens are most frequently from 60–65 mm. in length (about 90 per cent. of the community is between 50–70 mm. and less than 2 per cent. are over 70 mm. long). The widths most frequently range from 35–40 mm. Recently Professor Swinnerton has studied the variations in young

growth-stages of *Gryphæa incurva* from the type locality of Fretherne, Gloucestershire (1939, fig. 5). His graphical results have been superposed on the corresponding graphs for the Loch Aline community in fig. 5. It will be noted that in several of the graphs there is very close similarity. This is especially true as regards the angle of rise of the profile of the shell from the area of attachment and the ratio of breadth to length (fig. 5, *b*, *c*); in the latter case the graphs show a similar position of the mode, but the Fretherne specimens tend on the whole to be a little wider and to include fewer narrow specimens. As regards the height-breadth ratio there is only general similarity (fig. 5, *d*); the range of variation is similar, as in the last graph, but the Fretherne specimens appear to be somewhat broader in proportion. The length of the area of attachment also shows a similar range. As regards the smaller areas some difference in the graphs is probably due to the difficulty of measurement. It will be noticed, however, that there are very few areas of attachment greater than about 7 mm., although the range extends to a maximum of about 25 mm. in odd specimens of both communities.

The variation of the young growth-stages was studied on the basis adopted by Professor Swinnerton as already described, the stages chosen being of the same average size as those measured on the Fretherne specimens. The ratios of height to breadth and breadth to length (referred to by Swinnerton as the umbonal profile and the outline respectively) have been plotted as a scatter diagram (fig. 6) and the "scatter periphery" of the Fretherne scatter marked by broken lines (from Swinnerton, 1940, fig. 4). It will be noted that the main area of the scatter is common to the two diagrams but that there is some difference in the extent of the variation, owing to the varying range of the breadth-length ratios, the early breadth already noted in some Fretherne specimens being responsible for much of the difference. It must be remembered, however, that the form of the scatter periphery depends only on the characters of a few outlying shells of the community and a large proportion of the shells fall within those parts of the scatters which coincide. The difference in the ranges of these two scatters, however, representing measurements of young shells in communities of what may be regarded as the same species, is noteworthy and perhaps is significant. It is interesting to note that the Loch Aline scatter actually shows less difference from the scatter illustrated by Swinnerton for the shells of *Ostrea irregularis* from a lower horizon in South Wales.

In fig. 6 there has also been inserted the scatter periphery for a group of young *Gryphæa obliquata* from a lower horizon in the Bucklandi Zone illustrated by Swinnerton (Bli in fig. 10, 1940). It will be seen that this

scatter coincides almost exactly with the area of greatest density of points in both the Loch Aline and Fretherne diagrams.

Unfortunately Professor Swinnerton did not publish many scatter graphs showing variation in the adult members of the Fretherne community of *G. incurva*. It is, however, possible to make some comparisons with the results of measurements of the Loch Aline specimens.

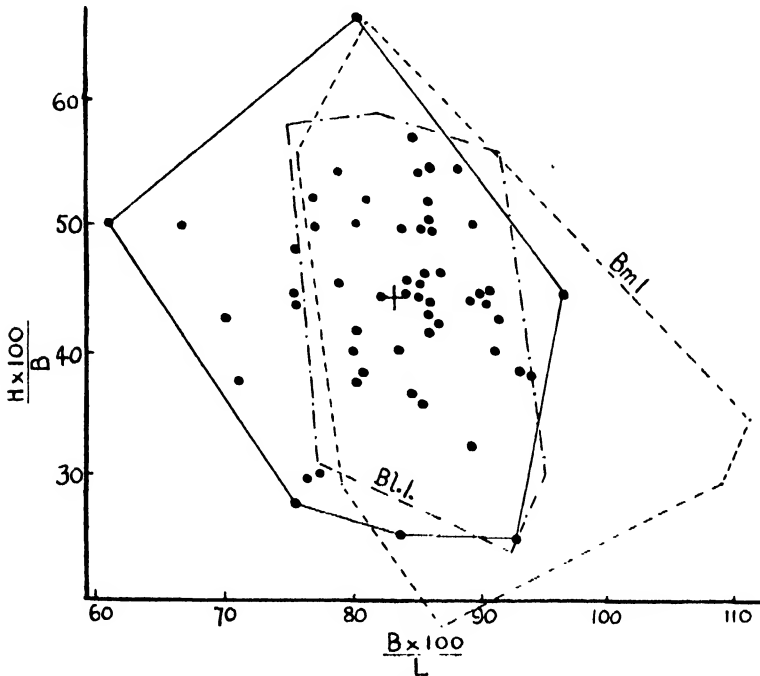


FIG. 6.—Scatter diagram of height-breadth and breadth-length for the young growth-stages of the *G. incurva* community from Loch Aline. The “scatter peripheries” for the corresponding features in a *G. incurva* community from Fretherne (Bm. 1) and in a *G. obliquata* community from Keynsham, Som. (Bl. 1), are inserted, after Swinnerton (1940, figs. 4 and 10).

The scatter diagram for breadth against length and the variation diagram for the ratio $\frac{B \times 100}{L}$ are shown in fig. 7. The ratio ranges from about 45 to 80 per cent. with a mode of 60–65. It may be noted that these figures are not strictly comparable with the breadth-length ratios illustrated in Swinnerton (1940, fig. 7) because he was referring to the length of the last-formed lamina of shell. Using his dimensions there is very little significant difference in the variation in these ratios in the adults of the two communities. On the graph (fig. 7) those forms which possess a marked sulcus have been marked with a cross. It should be

noted that many of these fall on or near the upper edge of the scatter, suggesting a tendency for the association of a stronger sulcus with the wider forms.

It may perhaps be noted that there is direct and close correlation between breadth and length, the points of the scatter approximating very generally to a straight line. Swinnerton in 1940 (p. c) spoke of the negative correlation of length, breadth, and height in a community of his specimens. It should be emphasised that the negative correlation to which he referred is for the ratios height to breadth and breadth

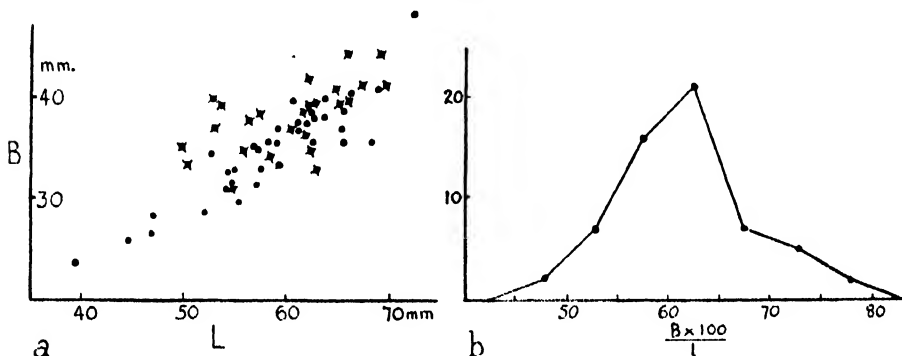


FIG. 7.—Scatter diagram (a) and variation graph (b) to show relation of breadth to length in the adults of the Loch Aline community. Specimens with a marked sulcus are indicated by crosses.

to length; there is obviously positive correlation between the actual measurements. The different position of breadth in these two ratios must necessarily augment the appearance of negative correlation.

The ratios of the total length of the shell to the length of the right valve are shown in fig. 8. The scatter forms approximately a straight line conforming generally to $l = .68 L$.

A similar scatter has been made to show the relation of height to length ($H - L$). Since these dimensions are two diameters of the spiral measured at right angles and assuming that the curve is a logarithmic spiral, the angle α can be determined from the formula $\cot \alpha = \frac{2 \log H/L}{\pi \log e}$.

On the diagram (fig. 9) lines have been inserted in the scatter for the $H - L$ ratios of a number of spiral angles. It may be noted that the middle of the scatter approximately corresponds with a spiral angle of 77 degrees, while most of the specimens range from 72 degrees to 81 degrees 30 minutes. This affords a general confirmation of the measurements of the spiral angle, but only a general agreement can be expected

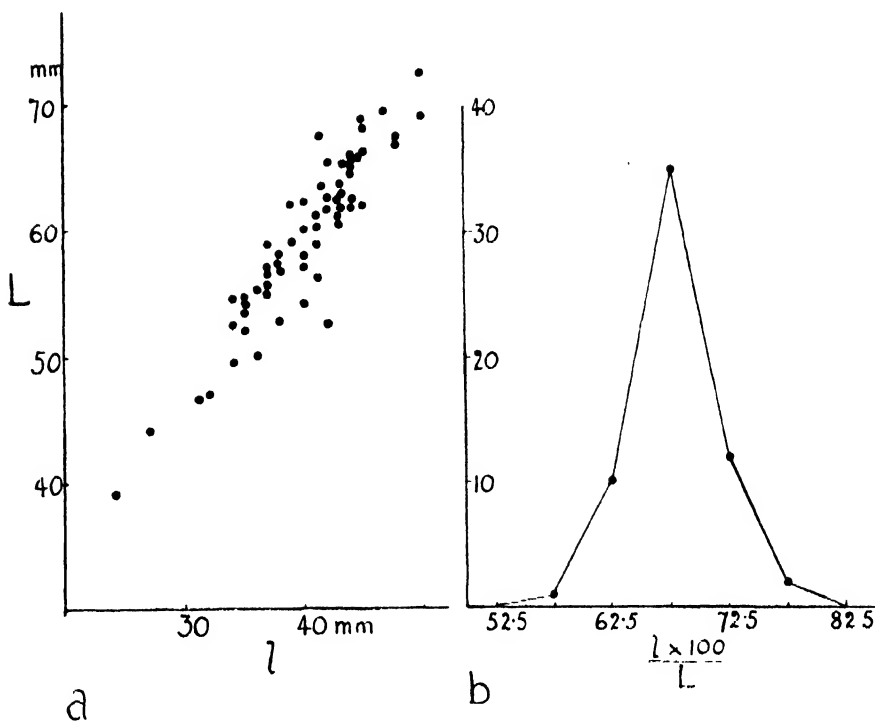


FIG. 8.—Scatter diagram (a) and variation graph (b) to show relation of total length (L) to length of right valve (l) in the adults of the Loch Aline community.

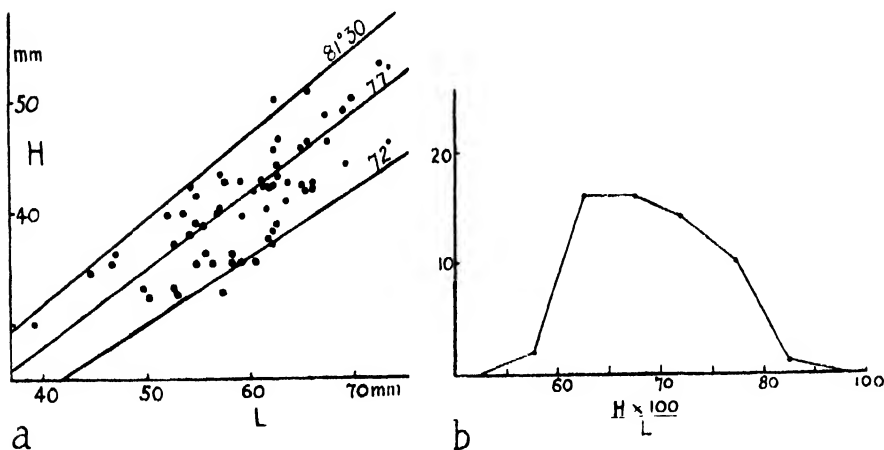


FIG. 9.—Scatter diagram (a) and variation graph (b) to show the relation of total length (L) to height (H) in the adults of the Loch Aline community. The spiral angles for corresponding equiangular spirals are inserted.

since the dimensions measured are affected by the changes in spiral angle which occur during growth.

The ratio of CL to l has also been plotted (fig. 10) and lines for various spiral angles have been inserted on the basis of the formula

$$\frac{\text{length of spiral}}{\text{radius}} = \sec a. \quad \text{Since}$$

CL is approximately the length of spiral and l approximately the radius (although in fact generally shorter than the radius) a rough calculation of spiral angle is possible. The points appear to correspond with spiral angles ranging from 67 degrees to 76 degrees, but owing to the lack of accurate measurement of the radius and spiral these angles are to be regarded as less trustworthy than those shown in fig. 9.

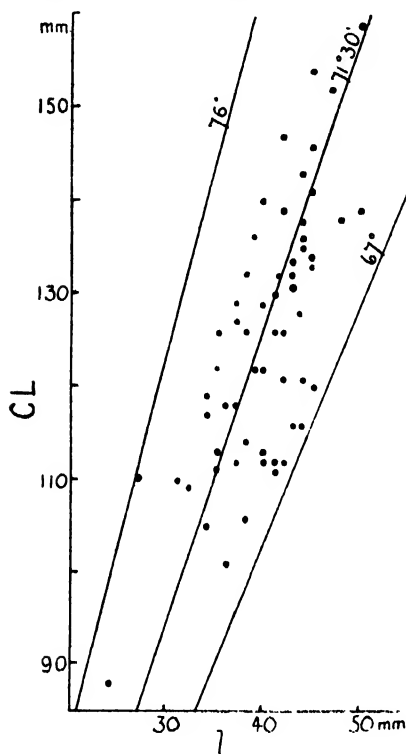


FIG. 10.—Scatter diagram to show the relation of the length of the curve of the left valve (CL) to the length of the right valve (l) in the adults of the Loch Aline community. The spiral angles for corresponding equiangular spirals (assuming that l is approximately equal to the radius) are inserted.

SULCUS.

The development of a sulcus is frequently much more pronounced in *Gryphæa incurva* than in *G. obliqua* and some other later *Gryphæas*. In its extreme development the sulcus represents a deep channel tending towards the separating of the posterior part of the shell. In the Loch Aline specimens the sulcus is very often well developed, and a deep

sulcus is present at some stage of growth in the majority of the specimens. The prominent sulcus is usually present by the time the shell reaches the length of 30 mm., but it rarely persists with any strength on the last quarter of a whorl; in some specimens it becomes completely obliterated in the final part of the shell. The sulcus tends to continue to the margin most frequently in those wider shells in which the spiral angle is small (compare fig. 7).

While in many specimens the sulcus forms a continuous channel, occasionally the line of the sulcus is broken, the channel being recom-

menced out of line with the earlier sulcus. In a few specimens more than one such break in its continuity is present.

A deep sulcus is usually associated with a steep bend on the growth lines. The shell border at the sulcate stage must have been bent back suddenly at the posterior margin. This feature may well have been related to a distinction between the inhalent and exhalent parts of the mantle border, the sulcus marking an inflection of the mantle separating these regions. It may be noted that where the sulcus fades out in the final growth-stages the growth lines tend to become straight (figs. 3, 11, *a*).

AREA OF ATTACHMENT.

The range of size in the area of attachment has already been noted (p. 217). Very frequently the larger attachment areas show evidence of the nature of the shell to which the specimen has been attached. In some cases the growth lines may represent a fragment of *Gryphæa*, but the attachment appears most commonly to have been to shells of *Oxytoma inaequivalve*. The area of attachment is usually nearly the same size as the shell. In most cases attachment has been to the outside of the shell, and the area of attachment shows a mould of the ornamentation and other structural details (fig. 11, *d*). In most of the specimens where such attachments are visible the right valve has in its umbonal region an exact counterpart of the area of attachment; the reflection of the *Oxytoma* shows details of ribbing so sharply that its identification presents no difficulty. It is apparent that up to the growth stage represented by the length of the area of attachment in these cases the shell margin of both valves was formed in close association with the surface

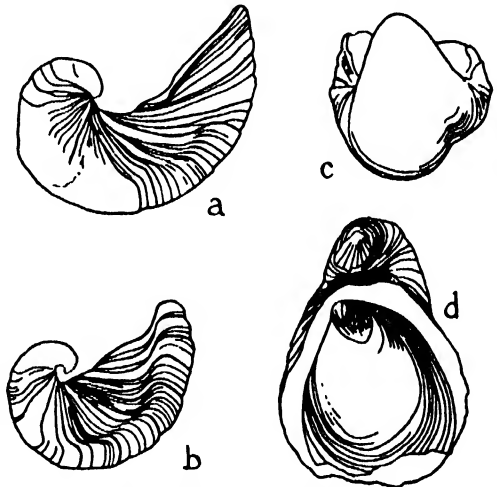


FIG. 11.—Specimens of *G. incurva* from Loch Aline. Slightly reduced. *a*, specimen showing a strong sulcus and bend of growth lines at the early adult stage, followed by a stage with no sulcus and simple growth lines (Hunterian Museum, No. S. 7743); *b*, specimen with strong sulcus throughout (Hunterian Museum, No. S. 7744); *c*, dorsal view of specimen showing depth of sulcus (Hunterian Museum, No. S. 7745); *d*, specimen showing attachment to shell of *Oxytoma inaequivalve*, and the "reflection" of the attachment on the right valve (Hunterian Museum, No. S. 7746).

of attachment. Similar features are often exhibited by oysters (*Ostrea irregularis*) in the Angulata Zone which have attached themselves to ammonites and in which the right valve shows a reflection of the solid form of the whorls (Lang, 1928). Jones illustrated a similar reflection of the pattern of the surface of attachment in the Gloucestershire shells which he described eighty years ago; in some of his examples the spat had become attached to adult Gryphæas (Jones, 1862, pl. ii, figs. 5 and 6).

It is obvious that the young Gryphæas developed as flattish oyster-like forms to various dimensions, some commencing to form an arched shell when they were not more than 2 mm. in length, others delaying that character until they were in rare cases over 20 mm. in length. In all of them, however, the development of the gryphæate form appears to have been marked by a thickening of the attached (left) valve accompanied by a sudden turning up of the ventral lip. That stage represents the initiation of the arcuate form and from that time a similar method of growth persists.

While many shells in the Loch Aline community show attached spat, sometimes attaining a length of several millimetres, apparently only a small proportion of the adults in the community commenced life by attaching themselves to shells of their own species. This is noteworthy, for it is unlikely that many other suitable objects for attachment, apart from occasional shells such as *Oxytoma*, were available on the muddy floor. It may be possible that many of the individuals which became attached in this way to older shells subsequently died.

THE EVOLUTION OF GRYPHÆA IN RELATION TO POSSIBLE ENVIRONMENTAL FACTORS.

It may be taken as established that gryphæate forms have been evolved from more normal oysters at several times during the Jurassic. Probably both environmental and hereditary factors have influenced this evolution. Several writers (for example, Lang, 1923, p. 11; Trueman, 1940, p. 81; Swinnerton, 1940, p. lxxviii) have acknowledged that at least in some stages environmental factors may have modified the shell form. Such factors may represent the stimulus which initiated the gryphæate trend or they may have controlled its development throughout. Slight differences in the conditions on the sea floor within an area of a few square yards may have led to some of the variations noticed above, especially in the young shells which might be expected to show more closely the influence of such differences. Probably the most important problem before the evolution of *Gryphæa* can be properly understood is

the disentanglement of the features due to the influence of the immediate environment on each individual from those which are due to inheritance; "before we can get an idea of the hereditary variation, we must eliminate [the] environmental factor as far as possible" (Waddington, 1939, p. 286).

As a preliminary to assessing the nature of environmental influences on *Gryphæa* some attention has been given to the mode of life of the modern oyster. This organism has been widely studied, and indeed has been claimed to be the best known marine animal in the world. The oysters of commerce have generally been reared in special conditions, having been broken from their original attachment and spaced on a suitable sea bottom in order to produce regular growth. Much more useful information can be gathered from oysters which have grown naturally in crowded situations and also from shells which have been transferred to environments of different character.

A very casual study of modern oysters makes it clear that the shape and size of the area of attachment varies according to the object on which the spat settled; in cases where the oyster had grown in attachment to the spire of a gastropod or to the bowl of a clay pipe (Kellogg, 1910, figs. 27, 52, 53) the area of attachment necessarily showed peculiarities very different from that produced when the spat had been attached to a piece of slate. In the latter (seen at Conway) the area of attachment was large, smooth, and in the plane of the valves, the shell being extraordinarily flat. It is impossible to avoid the conclusion that the area of attachment, its size and its relation to the subsequent shell, depend very largely on the nature of the solid object to which the spat became attached. If the spat became attached to a small solid object it is obvious that only a small area of attachment was possible. The influence of these factors on the shape of the young shell in *Ostrea edulis* has been emphasised by several writers (Orton and Amirthalangam, 1927, p. 947). In that species the attachment must be clean and free from slime, and the spat shows a preference for attaching itself to the under side of any objects where such attachments are available; thus a position beneath the lip of another shell is frequently chosen.

In subsequent growth the form of the oyster is also frequently affected by its surroundings. If a large number of spat have attached themselves to one object the growing shells become crowded and tend to assume long narrow forms. If, however, such crowded and misshapen young forms are separated and given a situation where they have ample space they tend to acquire a normal shape (Kellogg, 1910, pp. 238-9). Indeed Orton has recently suggested that the form of an oyster shell may be "entirely a product of its environment" (1937, p. 15). This can be

illustrated in several ways. The modern oysters broadly form two main groups. The first contains the English oyster (*O. edulis*), a flat form; the second contains the Portuguese and American oysters (*O. angulata* and *O. virginica*), which have a somewhat arched (gryphæate) type of shell. The latter can live in more turbid water and can tolerate a greater degree of muddiness than the flat types of oysters. It is recorded, however, that the Portuguese oysters bred on English oyster beds (under conditions suitable for *Ostrea edulis*) tend to resemble the English oyster in shell form (Orton and Awati, 1926, p. 228). One of us was shown at Conway Oyster Breeding Station examples of shells of *O. edulis* which had grown normally for several years and then, being transferred to soft ground in the Menai Strait, had developed a sharp bend on the left valve and a tendency towards a more cup-shaped form of shell. Dall (1898, p. 675) has described similar examples of environmental influence.

These features must be related to the way in which the oyster holds its mantle edge (Orton, 1928, p. 418) by which the shell margin is secreted and its form determined. The position of the mantle edge is in turn influenced by the feeding habits and environment, the latter exercising a considerable influence on the form (Orton and Amirthalingam, 1927, p. 948). Accounts of the feeding habits of oysters have been given by Yonge (1926) and Nelson (1938). For the present purpose, attention may be directed mainly to their conclusions regarding the importance of the mantle which, according to Nelson, forms pallial curtains opened along the margin only in restricted regions for entrance and egress of water; it is important for the oyster to be able to change the position of these entrances in order to maintain itself on a loose and shifting sea bottom. Nelson records that after a gale large oysters of 12–14 cm. in length were found buried hinge downwards with only 2–3 cm. of their free margins projecting and with well-defined channels free of mud along their inhalent and exhalent margins (Nelson, 1938, p. 35).

It has already been suggested that *Gryphæa* is a form adapted to life in a situation where there was more mud than in that occupied by typical Jurassic oysters (Trueman, 1940, p. 81). It may be presumed that the left (arched) valve was downwards in the mud with the ventral margin projecting upwards. The developing *Gryphæa* must at a comparatively early stage have become free and able to take up this position. There are probably two ways in which it may have become free. In the first place its attachment may have been to some light or small object so that the growing shell began to tilt gradually as its weight increased and as it disturbed the balance of the small object on the loose sea floor. In

the second place, if the oyster were attached to some more stable object, it may be supposed to have broken free at some stage when its weight and the distance of its centre of gravity from its attachment made that possible; the shells attached to a stable object which grew without breaking loose would frequently be at a disadvantage in any muddy environment and many of them would ultimately perish.

In a small area of the sea floor there would obviously be a wide range of situations available to *Gryphæa* spat in which their subsequent growth would be controlled by a great variety of factors; changes due to increasing or decreasing muddiness in successive seasons would add still more variety. Much of this diversity of environment would be peculiar to attached or sedentary small organisms and the adult *Gryphæas* in a similar area would live under conditions which were much more nearly uniform, save for changes possibly of a seasonal or periodic nature, in an area where mud was effectively accumulating.

In the opinion of the writers the young *Gryphæa* inherited wide powers of variation in relation to the particular environment in which it developed. It appears to be legitimate to draw such a conclusion from the evidence available in the development of the modern oysters; it seems more likely that these variations are due to direct environmental influences than that they were produced by differences in the inheritance of the various individuals.

After this early stage in which the developing shell was greatly influenced by the size and position of the object to which it was attached, there followed a period of growth in which it may be presumed that the shell was lying with its left valve in the soft mud of the sea bed. During this stage the shell-form approximated to a logarithmic spiral, shell growth being predominantly upwards at the ventral margin; this growth would have the effect of keeping the shell margin above the level of the mud, even though new deposits were accumulating or the shell had a tendency to sink deeper into the mud. Probably the additional deposit of calcareous material under the left umbo balanced to some extent the upward growth of the margin. During this stage also it appears that a marked sulcus separated the inhalent from the exhalent portions of the mantle border, there being a tendency for the inhalent portion to be built up more rapidly (perhaps to keep it above the mud). As the shell became bigger the frequent disappearance of the sulcus suggests that this distinction may no longer have been necessary in all cases, probably because the margin of the larger shell was sufficiently raised.

There are many difficulties to be faced in any attempt to distinguish between those features in the variation of *Gryphæa* which may be regarded

as purely environmental and those due strictly to some inherited character. Sufficient has been said to support the view that many features in the growing *Gryphæa* may represent the influence of the environment on an organism which was capable of considerable variation as regards shell-form. It would appear that many of the differences to be found in the juvenile stages of the *G. incurva* community at any locality may be due to differences in the sea floor; it is also suggested that the differences in the range of variation in those juvenile stages in different localities may merely reflect slight differences in the environment; subsequent growth appears to indicate that in each case the juvenile stages develop into essentially similar adult forms.

While admitting the importance of these environmental factors, however, it is clear that in the evolution of the *G. incurva* lineage certain changes in the inherited factors must have taken place. Put simply, it is suggested that the spat of *G. incurva*, if introduced into the normal habitat of typical flat oysters, would not have produced adults of the ancestral type; if unable to live in that habitat in the gryphæate condition the forms would probably not have survived.

This change in the hereditary equipment of the lineage possibly involved the inheritance of an increasing tendency to coil. This appears to be supported by several facts.

- (1) Although the *G. incurva* lineage shows progressive development of the gryphæate character, there was not a corresponding increase in muddiness during the period through which the lineage lived. In South Wales, where the commencement of the gryphæate coiling is so well displayed, its initiation corresponded with a sudden increase in muddiness (represented in the shales of the Lower Angulata Zone) which probably exceeded that at many subsequent stages in the history of the lineage.
- (2) In South Wales and Somerset, Gryphæas are found both within and outside the limits of littoral deposits, but the obvious difference in habitat does not appear to have affected the average curvature at any horizon: indeed, the shells can be used to correlate the littoral with the more normal development.
- (3) Assuming that *G. incurva* is directly related to *G. arcuata*, the lineage was widely distributed in Europe during the deposition of the Angulata and Bucklandi Zones. Typical incurved forms occur only in the later part of this time but they are found in a great variety of areas, and it is almost certain that the degree

of their curvature was not strictly related to the amount of muddiness in any particular locality.

It may be noted that the development of these incurved shells with thick deposits of calcium carbonate must have necessitated some modification of the calcium metabolism in the later members. It may be suggested that the ratio of the bulk of the organism to the total bulk of its shell would be an interesting subject for study. It is rarely easy to remove the material filling the body cavity in a complete shell, however. There is no doubt that the ratio of shell to organism increased progressively both during development and during the evolution of the gryphæate condition. As to the members of the *G. incurva* community, it may be wondered whether the more incurved forms, in which the difference in relative bulk is most pronounced, represent long-lived individuals which grew more slowly. A somewhat similar comparison may be made between the so-called "dumpy" oysters and normal oysters of some British oyster beds (Orton, 1936).

THE GENUS GRYPHÆA.

In 1938 Nelson re-emphasised a distinction which had already been made by some workers on living oysters, noting that two groups could be recognised among them. The first group includes *Ostrea edulis* and other species which are flat and which incubate larvæ in the mantle cavity. The second, including the Portuguese and American oysters (*O. angulata* and *O. virginica*), are cup-shaped, or arcuate, and do not incubate their larvæ. Nelson added to these distinctions another of very considerable importance. He showed that in the latter group there exists a promyal chamber on the right side of the organism between the dorsal (or anterior) region and the adductor muscle. Through this promyal chamber much of the water current passes. The flat oysters do not possess such a promyal chamber, the adductor muscle being in contact with the visceral mass.

Nelson believes that this development of a promyal chamber represents an adaptation permitting life in more turbid waters than the English oysters can withstand. There is no doubt that these cup-shaped oysters do live in much more turbid waters.

Nelson proposed to follow other workers in assigning the modern cup-shaped oysters to the genus *Gryphæa*. It is true that when introducing the genus Lamarck included the species *O. angulata* and this has been frequently regarded as the genotype of *Gryphæa*. We are informed by Dr L. R. Cox, however, that this species was not available for selection as a genotype since it had not been figured or described

when the genus was named. W. H. Dall (1898, p. 673) selected *G. arcuata* Lamarck as a genotype, a choice which is in conformity with the normal palæontological use of *Gryphæa*. Since *G. arcuata* is almost certainly a member of the *G. incurva* lineage this genus may be used with little doubt for the forms discussed here; its extension to cover other gryphæate oysters in the Jurassic is probably permissible, though it must be recognised that in that case the genus is polyphyletic. *Lio-gryphæa*, proposed by Fischer (1886, p. 927) with *G. arcuata* Lamarck as genotype, is thus a synonym of *Gryphæa*.

Nelson has already stated that the Jurassic Gryphæas are not likely to have possessed a promyal chamber. The presence of this chamber necessitates the movement of the adductor muscle towards the ventral (or posterior) border of the shell. In Jurassic Gryphæas the muscle scar appears to have been nearly central, a position very similar to that in normal oysters. It therefore appears that the Jurassic Gryphæas were not equipped in the same manner as the modern cupped oysters to deal with excessive muddiness; the sudden disappearance of *Gryphæa* above the limestones at Loch Aline may well be related to the excessively muddy conditions which followed.

If these views as to the relation between *Gryphæa* and the various modern oysters are correct it follows that the Jurassic Gryphæas represent off-shoots from the main oyster stock modified for life in somewhat muddy situations. A different type of adaptation has been carried still further by the modern cupped oysters. It is clear, however, that these cannot be placed in the genus *Gryphæa*, and if it is desirable to separate modern oysters into two genera, then a different name is required for the cupped forms; Dall (1898, p. 671) suggested that *Crassostrea* Sacco is suitable.

SUMMARY AND CONCLUSIONS.

The variations of the adult shells of the *G. incurva* community at Loch Aline are essentially similar to those at Fretherne, Glos. All show a moderate degree of curvature of the shell, and while some of the variations may represent adaptations to the slightly varying conditions on the sea floor, it is suggested that the group was living under fairly uniform conditions in a somewhat muddy environment.

On the other hand, it is suggested that many of the variations in the juvenile stages may be quite fortuitous, depending on the variety of situations available to small attached shells, and particularly upon the size, nature, and situation of the object to which the young individual became attached. The different range of variation in the juvenile stages

of communities in different areas may thus be related to these environmental factors, rather than to any essential difference in the hereditary constitution. Caution is necessary in interpreting developmental variations in a group where the young were attached and exhibited unusually wide plasticity. Detailed studies on other *Gryphæa* communities may make it possible to separate features due to direct adaptations of individual juveniles from those changes which more permanently affected the lineage, but at least the need for this distinction must be kept in mind.

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232 *Gryphæa incurva* (Sow.) from the Lower Lias of Lock Aline

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(Issued separately May 5, 1942.)

XIX.—pH Phenomena in Relation to Stomatal Opening: II-V.
 By **Professor J. Small, D.Sc., Miss M. I. Clarke, and Mrs J. Crosbie-Baird**, Queen's University, Belfast. (With Nine Text-figures and Two Tables.)

(MS. received December 30, 1941. Read May 4, 1942.)

CONTENTS.

	PAGE
Part II. SOME FACTORS IN STOMATAL MOVEMENTS, by J. SMALL	233
Part III. OX-EYE DAISY AND OTHER SPECIES, by J. SMALL and M. I. CLARKE	236
Part IV. TIME RELATIONS OF STOMATAL MOVEMENTS, by J. SMALL and M. I. CLARKE	247
Part V. TIME AND PH RELATIONS IN AMYLASE ACTIVITY, by J. SMALL and J. CROSBIE-BAIRD	254

Part II. Some Factors in Stomatal Movements.

By Professor J. SMALL, D.Sc.

THE control of stomatal aperture by intensity of light, under normal conditions of water-relations of surrounding cells, has apparently been established by Nutman (1937) for coffee leaves. This control results in the closing of stomatal pores both with too little and with too much illumination. Since photosynthesis in the *guard-cells* is not known to decrease with increase in light intensity, up to the limit of full sunshine, some other factor or factors are suggested as coming into action.

Investigations, in the Department of Botany, Queen's University, Belfast, by three research students have progressively elucidated some of the factors concerned.

Miss K. M. Maxwell (see Part I, Small and Maxwell, 1939) by careful observation collected the data for coffee and some other species. Her results show that there is normally both an opening and a closing of stomatal pores within the natural range of internal pH of the guard-cells under conditions of darkness and of light. One of the outstanding features of these data is the demonstration that the optimal pH for stomatal opening depends to some extent upon the kind of buffer system which is used in the external fluid medium. These optima for coffee are—in acetate pH 5·7, in phosphate pH 6·6, which correspond closely

with the optima for the hydrolysing action of saliva-amylase, pH 5.6 acetate and pH 6.6 in phosphate buffer solutions. The similar difference found with optimal pH values for stomatal opening was a mystery to the student until the corresponding differences in amylase effects were pointed out by the present writer.

Since photosynthesis, by removing carbon dioxide from solution within the guard-cells, could raise the pH beyond the optimum, the resulting decrease of stomatal aperture with high insolation might explain Nutman's observations on reduced photosynthesis connected with stomatal closure in bright sunshine. The time-lag of about two minutes or more which he observed is commonly regarded in botanical circles as too short for effective enzyme-action, but this also has been investigated with positive results. Biochemists are well acquainted with rapidly effective enzyme-action in salivary digestion of starch, in muscular movements, etc., where the enzymes act at least as quickly as they do in the "flash blueing" of *Boletus* tissue.

Miss M. I. Clarke in a further series of investigations (Part III of this series) has corroborated the previous observations on ten species and extended our knowledge of similar phenomena to six other species. She has also corroborated observations on the decrease or disappearance of starch as correlated with stomatal opening, and the increase or appearance of starch as correlated with stomatal closure. The present writer "discovered" a close negative correlation between average stomatal aperture and average starch content, in data which had been regarded as "hopeless" by the above research student.

In another investigation (Part IV of this series) Miss Clarke has observed the time-relations of stomatal opening and closure. She finds a minimum of two and a half minutes, in phosphate buffer solution, for partial opening from the fully closed condition; and a minimum of thirty seconds, in citrate buffer solutions, for complete closure from the fully open condition. Closing was observed in most cases to occur more rapidly than opening.

These results would appear to indicate that rapid enzymic action is at work in the mechanism of stomatal movements, the guard-cells opening the pores more slowly against the mechanical opposition of guard-cell shape and closing the pores more rapidly when acting in conjunction with the natural shape of the guard-cells, which shape occurs in the closed condition.

While this research was proceeding, a third-year student reported an anomalous result with the usual time-test for the disappearance of starch under the action of malt diastase. This student, now Mrs Joan

Baird, has continued investigation of the phenomena reported. She has obtained data (Part V of this series) showing that the starch-iodide test fails within two or three minutes of a particular starch solution being mixed with an amylase solution at particular pH values. Since the failure of the starch-iodide test is no final proof of sugar-formation or of increased osmotic pressure, the same student has further determined quantitatively the reducing power (as "maltose") present in such a mixture after short intervals of enzymic action from two minutes upwards. She finds that with enzyme-action of only two minutes duration there is a notable increase in the reducing substances at certain critical pH values.

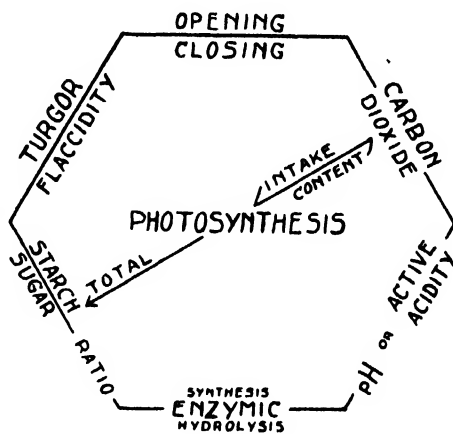


FIG. 1.

As an introduction to the presentation of these observations in more detail, we can consider the scheme suggested in fig 1.

Light is omitted as a distinct factor because it is implicit in the term "photosynthesis." Starting with the central photosynthesis—this in the guard-cells controls their carbon dioxide content,

which controls the pH or active acidity,

which controls the "amylase" or enzymic activity,

which controls the sugar/starch ratio,

which controls the turgor,

which controls the stomatal aperture,

which controls the intake of carbon dioxide by the leaf for general photosynthesis.

The factors on the outside of the hexagon are positive for opening—enzymic hydrolysis, increased sugar and other reducing osmotically active substances, increased turgor, increased opening. Photosynthesis in the guard-cells would increase the total carbohydrate available. The factors on the inside of the hexagon are positive for closing—enzymic synthesis, increased starch or decreased sugar, increased flaccidity, increased closure or decreased aperture.

Hanes points out (1937, pp. 202 and 210) that the degradation products of starch, especially with α -amylase, may give an eight- to nine-fold increase of osmotic pressure, even when freed from any sugar produced

during the breakdown of starch. The enzyme system in the above scheme possibly or even probably includes enzymes of the phosphorylase type obtained by Hanes from potatoes (1940 *b*) and peas (1940 *a*). The synthetic part of the scheme, formerly rather theoretical, has now been given a sound experimental basis by Hanes, who writes (1940 *a*, p. 422): "Of considerable botanical interest is the fact that the conversion of starch to glucose-1-phosphate is a reversible reaction; this is catalysed in both directions by an enzyme which will be referred to as a phosphorylase." Actual grains of starch were obtained by phosphorylase synthesis in later experiments by Hanes (1940 *b*), and on the time-relations of enzymic action on his synthetic starch he has a significant sentence: "The iodine-colouring property is rapidly destroyed by the action of α -malt or salivary amylases."

Part III. Ox-Eye Daisy and Other Species.

By Professor J. SMALL, D.Sc., and Miss M. I. CLARKE, M.Sc.

The methods used were essentially similar to those used by Small and Maxwell (1939). The same strip of leaf-epidermis was examined at half-hourly intervals for two and a half hours, yielding five observations of apertures at each pH used. A different strip was taken for each of the several pH values used. Five epidermal strips were used at each pH value for observations of the starch content at time-intervals corresponding to the five intervals used for aperture observations, yielding five records of starch content at each pH value used. The buffer-solutions used were of citrate, acetate, phosphate and borate systems, usually with the pH adjusted in 0.2 steps.

The apertures were again estimated approximately—as 3 for fully open, 0 for fully closed, with intermediates 2 for about half open and 1 for nearly closed. This rough estimation has already yielded valuable data, see Small and Maxwell (1939).

The starch content was given a numerical value for averaging; 2 for obvious starch, 1 for a little starch, and 0 for no starch seen in the guard-cells when the strip was tested by flooding with iodine and potassium iodide solution. This very rough estimation has yielded the consistent average results shown in the graphs and data given here.

The natural range of pH within the guard-cells in light and in darkness was determined, using Small's Range Indicator Method (Small, 1929). This range may vary with different material of the same species, and observations were repeated on the species previously reported on by Small and Maxwell (1939). The natural ranges found in the present

investigation are given below, with the previously published ranges in brackets. The higher pH values occur in light, the lower in darkness.

<i>Coffea arabica</i>	5.6 to 4.0 (6.2-5.9 to 5.2-4.4)
<i>Kniphofia</i> sp.	5.2-4.4 to 4.0 (5.2 to 4.4)
<i>Vicia faba</i>	5.6-4.8 to 4.4 (5.6 to 5.2-4.8)
<i>Narcissus pseudonarcissus</i>	5.2-4.4 to 4.0 (5.2-4.4 to 4.0)
<i>Iris pseudacorus</i>	5.2-4.4 to 4.0 (5.2-4.8 to 4.4)
<i>Centranthus ruber</i>	5.2-4.8 to 4.4 (5.2-4.8 to 4.4)
<i>Hyacinthus</i> sp.	5.2-4.8 to 4.0 (5.2-4.8 to 4.0)
<i>Rumex obtusifolius</i>	5.2-4.8 to 4.0 (5.6-4.8 to 4.4)
<i>Tulipa</i> sp.	5.2-4.8 to 4.0 (5.2-4.8 to 4.0)
<i>Bryophyllum calycinum</i>	4.4 to 4.0 (4.4 to 4.0)

Observations on the stomatal apertures of the above plants in relation to pH yielded results very similar to those already published (Small and Maxwell, 1939). The similarity is so strong that they need not be republished.

The data for *Chrysanthemum leucanthemum* (the ox-eye daisy) and for five other species were tabulated as shown in Table I (see p. 239) which is for the first species only. In that table SA indicates stomatal aperture as estimated at half-hourly intervals on the same strip for each pH value, while Sh indicates starch content as estimated on a separate epidermal slip for each observation.

In these separate observations there is frequently an obvious negative correlation between extremes in stomatal aperture and extremes in starch content. There are, however, some odd cases and many intermediate conditions which are somewhat confusing in this tabulated presentation. By adding the numerical values assigned to each set of data and dividing by five, we obtain what we call (*a*) the average stomatal aperture and (*b*) the average starch-content. These values can then be plotted for each buffer-medium against the pH range investigated, and are here presented as figs. 1 to 6, with the natural pH range for each species indicated by vertical dotted lines. All the graphs in figs. 1 to 6 are derived from tabulations similar to those in Table I.

GENERAL RESULTS.

By reversing the scale for average starch-content as observed at various pH values, and placing the resulting graphs in proximity to those for average stomatal aperture as observed in separate series of epidermal strips, the general result becomes perfectly clear. Both average stomatal

aperture and average starch-content vary with the pH value of the surrounding medium, and these variations are correlated to a degree which is demonstrated by the similarity and parallelism of the graphs for the two sets of data. Mathematical treatment of the degree of correlation would be out of place considering all the rough approximations used to obtain numerical values for the data.

The general similarity of the pairs of graphs can be explained quite adequately on the basis of the diagram in the previous part (Small, Part II of this series). The pH and the type of buffer control the enzymic activity which controls the starch-sugar ratio which controls the turgor of the guard-cells, which turgor controls the stomatal aperture.

The first paper of this series presented evidence that average stomatal aperture varied over a wide range of pH, and that this variation included both opening and closing within the natural range of pH for the guard-cells. Some observations on coffee leaves were included concerning the presence or absence of starch in pH zones which varied with acetate and citrate buffers so that little or no starch coincided with the zone of maximum stomatal opening in each kind of buffer-medium.

The present investigation extends the starch data considerably, not only to other species and over a wider range of pH values, but also in a roughly quantitative measure to an obvious correlation between the degree of stomatal opening and the degree to which starch is decreased. The divergences from general similarity in the pairs of graphs are considered later.

Other general results include a repetition of former observations that in the natural pH range for the guard-cells, both opening (above SA 1.5) and relative closure (below SA 1.5), are found in practically all species with at least one of the buffer media used. The peaks or zones of distinct opening of the stomata vary also for the same species when different buffers are used, as demonstrated previously for the first ten species (Small and Maxwell, 1939). These and other points of interest are noted below for each of the present six species.

Chrysanthemum leucanthemum.—Table I and fig. 1. Natural pH range, light and darkness, 5.2–4.8 to 4.4. Citrate openings at pH 4.5 to 4.7, also 1.2–1.8, 2.4–3.9, and 5.6; closing maximum at pH 5.0, other approaches to closure at 2.0–2.2, 4.0–4.2, 4.9, 5.2–5.4, 6.0–6.7. Acetate openings at pH 5.7 (5.4–6.0), also 3.8; closing at pH 4.6 (4.1–5.2), also 3.0–3.2 and 6.4. Phosphate openings at pH 4.8, also 5.8–6.9 and 7.4; closing at 5.0–5.6, also 7.0–7.2 and 7.7–8.0. Borate opening at pH 8.9 (8.6–9.0); closing 9.2–10.0.

Divergence in the average starch-content occurs in citrate between

TABLE I.—CHRYSANTHEMUM LEUCANTHEMUM.

[illegible]

pH 3.0 and pH 4.0, where starch is at zero but stomatal aperture shows a gradual decrease. This might be due to the formation of less osmotically active amyloextrins. Divergences occur also between pH 1.0 and pH 2.0, and in alkaline borate zone. These may be connected with biocolloid

swelling or other subsidiary effects, but are well outside the natural range of pH within the guard-cells.

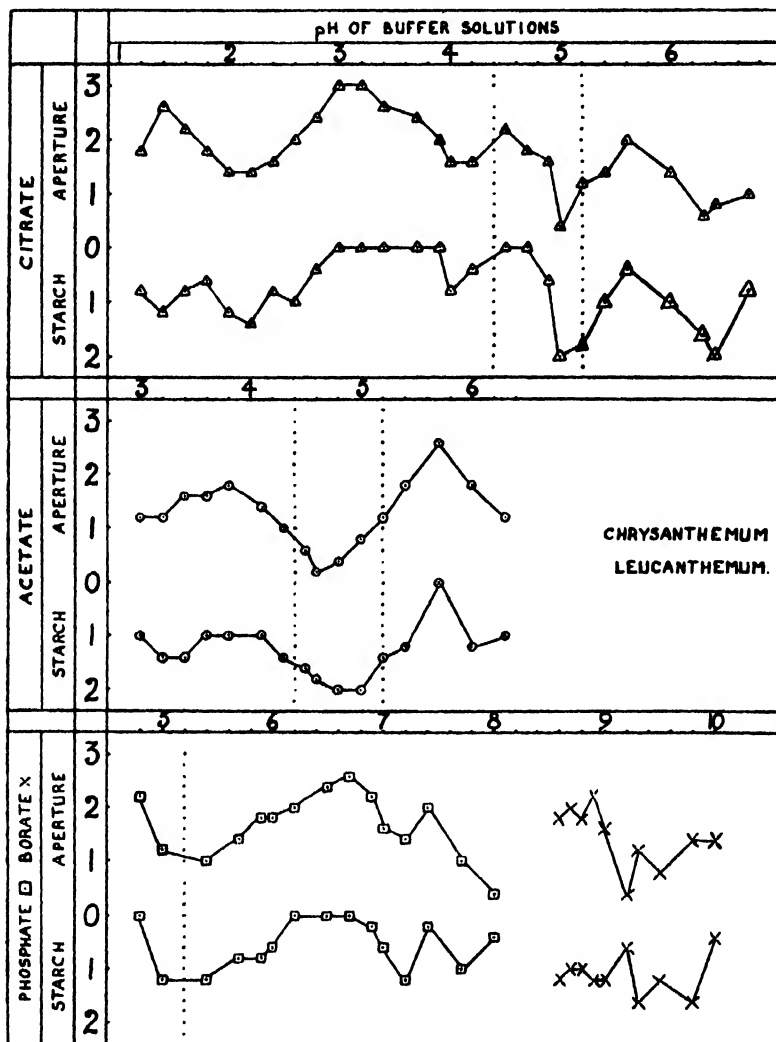


FIG. 1.

Beta vulgaris.—Fig. 2. Natural pH range, light and darkness, 4.4 to 4.0. Citrate openings at pH 4.0 (3.9–4.2), also 2.0–2.2, 5.2–6.0, 6.7; closing at pH 4.5 to 5.0, also 2.4–3.7 and 6.3–6.4. Acetate openings at pH 4.3–4.5, also 5.7 (5.2–6.0); closing at pH 3.0 to 4.1, also 4.6–5.0 and 6.3. Phosphate opening at pH 6.7 (6.5–7.0); closing over most of the

range 4.9 to 8.0. Borate opening at pH 9.0 (8.8-9.6); closing on both sides of this peak.

There is no marked divergence in the average starch-content here, except perhaps a flattening at "full starch" in citrate with decreasing

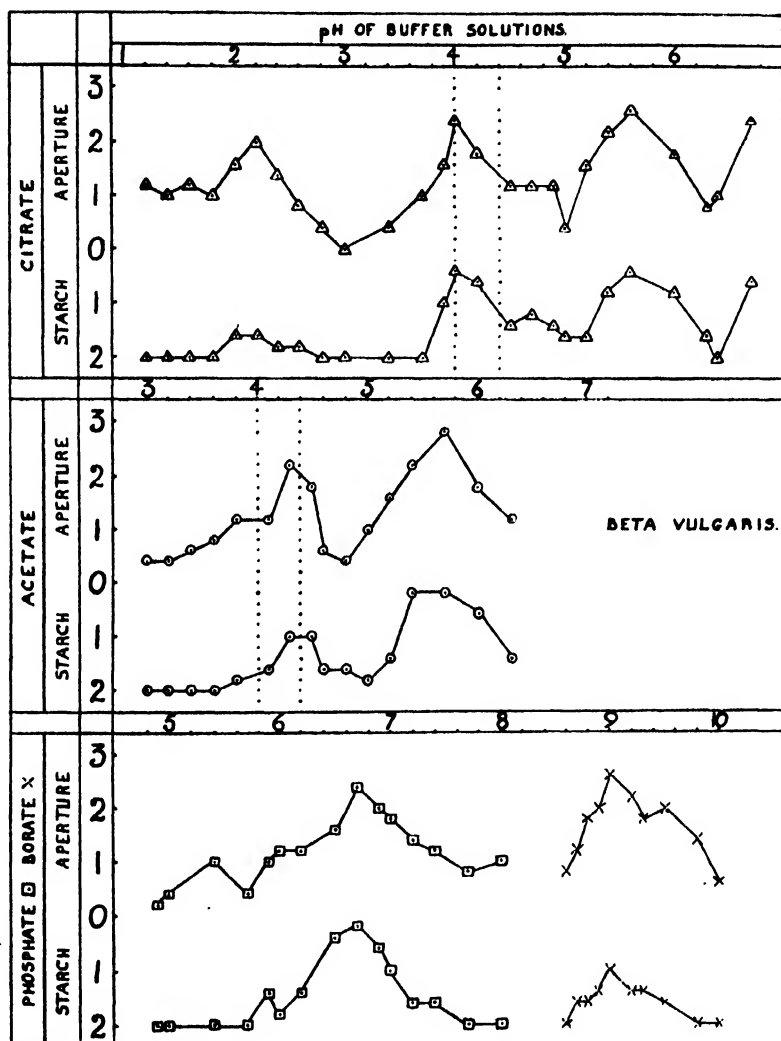


FIG. 2.

and increasing aperture between 2.8 and 3.7. This could be due to observational methods, as "full starch" allows for considerable variation in total quantity. The same explanation would hold for the flatness at

“full starch” in phosphate between pH 4·9 and 5·7; and for other similar cases given below.

Inula helenium.—Fig. 3. Natural pH range, light and darkness, 5·2–4·8 to 4·0. Citrate openings at pH 4·5 (4·2–4·7), and also 2·2–2·8

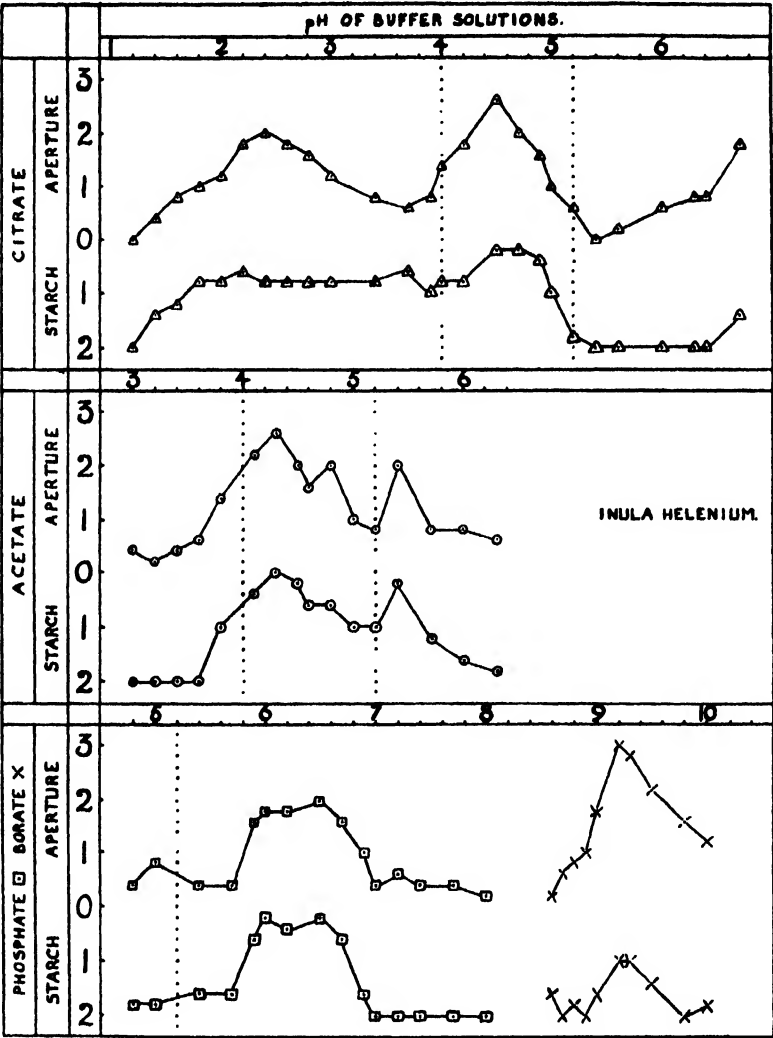


FIG. 3.

and 6·7; closing 3·0–4·0 and 5·0–6·4, also 1·2–2·0. Acetate openings at pH 4·1–4·8, also 5·4; closing at 5·0–5·2, also 3·0–3·8 and 5·6–6·3. Phosphate opening between pH 5·9 and 6·7, with closure on either side

of this peak. Borate opening between 9.0 and 9.5, with closure elsewhere in the borate range.

The small divergences in the average starch-content variation seem

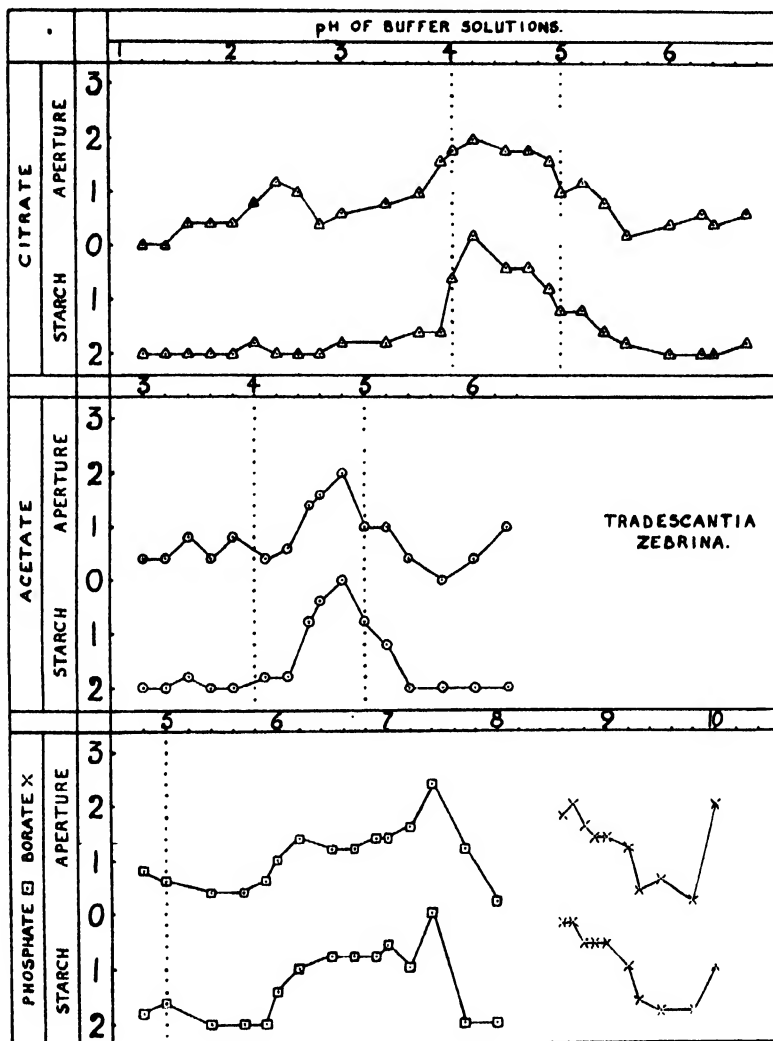


FIG. 4.

to be negligible in this case. They are well within the limits of observational errors.

Tradescantia zebrina.—Fig. 4. Natural pH range, light and darkness, 5.0–4.8 to 4.0. Citrate opening between pH 3.9 and 4.9, variations towards closure (below 1.5) on either side of this range, from pH 5.0

to 6.7 and from pH 1.2 to 3.7. Acetate opening from pH 4.6 to 4.8, and closing to various degrees elsewhere from pH 3.0 to 4.3 and from 5.0 to 6.3. Thus in both citrate and acetate the only openings are in the

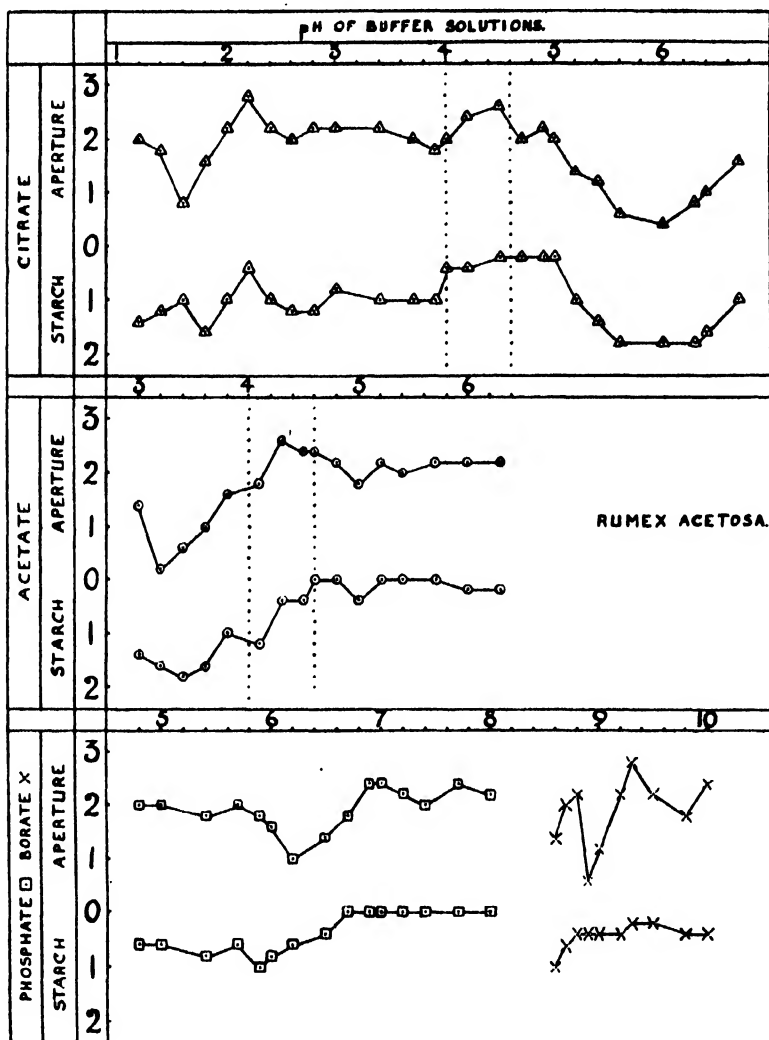


FIG. 5.

natural pH range, which also includes some degree of closure. Phosphate opening at pH 7.2–7.4, with values near or below “almost closed” elsewhere in the range pH 4.8–7.0 and 7.6–8.0. Borate openings at pH 8.6–8.8 and 10.0, with closing between pH 8.9 and 9.8.

Here there are some flat parts in the average starch-content at “full

starch," with slight variations in the average stomatal aperture. These "observational flats" at "full starch" may be explained as for *Beta* above. There seem to be no other significant divergences in this case between average stomatal aperture and average starch-content.

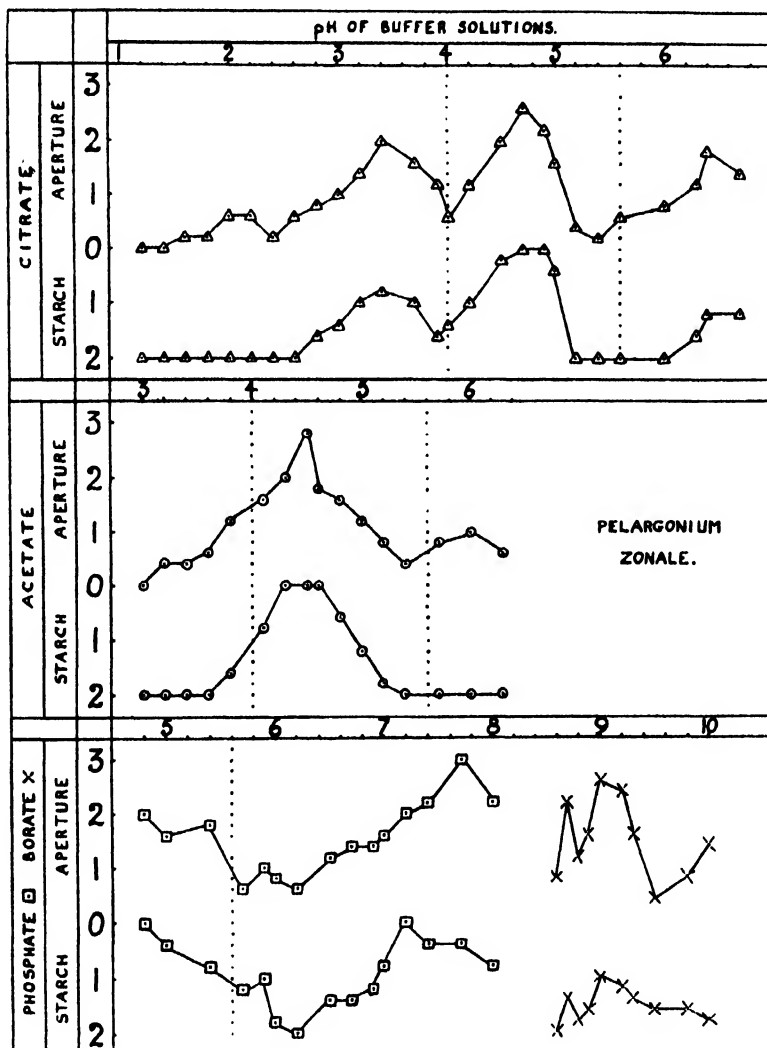


FIG. 6.

Rumex acetosa.—Fig. 5. Natural pH range, light and darkness, 4.6–4.2 to 4.0. Citrate opening over a wide range pH 1.8 to 5.0, with maxima at pH 4.5 and 2.2, also from 1.2 to 1.4; closing at pH 1.6 and from 5.2 to 6.4. Acetate opening from 3.8 to 6.3 with a peak at 4.3–4.6;

closing at pH 3.2, with variations towards closure between 3.0 and 3.6. Phosphate opening from pH 4.8 to 6.0, also 6.7 to 8.0; variations towards closure between pH 6.7 and 6.2. Borate openings at pH 8.7–8.8 and 9.2–10.0; closing pH 8.9–9.0, also 8.6.

These results are exceptional in that no marked degree of closure is observed in the natural pH range with either of the buffers used in that range. Citrate and acetate include the natural range, but this is outside the range of both phosphate and borate. In both cases there is a variation towards closure with lower pH values within the natural range, but this degree of closure does not reach our arbitrary value for "closed," 1.5 in average stomatal aperture. The tabulated data show that the open or half-open condition is reached quickly and persists for at least two and a half hours throughout the natural range, pH 4.6–4.0. In view of the general parallelism of the average starch-content, except in the borate medium, this species requires further investigation.

Pelargonium zonale.—Fig. 6. Natural pH range, light and darkness, 5.6–4.8 to 5.2–4.0. Citrate opening from pH 4.5 to 5.0, also 3.4 to 3.7 and 6.4; closing from pH 3.9 to 4.2, also from 5.2 to 6.0, with other variations towards closure from pH 1.2 to 3.2. Acetate opening from pH 4.1 to 4.8, with a peak at 4.5; closing on both sides of this peak from pH 3.0–3.8 and 5.0–6.3. Phosphate opening from pH 4.8 to 5.4, also 7.0 to 8.0; closing above pH 5.4 up to 6.9. Borate opening at pH 8.7, also 8.9 to 9.3; closing at pH 8.6 and 8.8, also 9.5 to 10.0.

Apart from "observational flats" at "full starch" in citrate and acetate, there seem to be no significant divergences in this case between average starch-content and average stomatal aperture. There is a slight divergence in the upper borate zone, but it does not appear to be significant for present purposes.

SUMMARY.

1. In the six species examined, stomatal opening varies with two factors—(a) the actual pH of the fluid in which the epidermal strips are immersed, and (b) the kind of buffer substance used.

2. The acetate maximum opening about pH 5.6–5.7 and the phosphate maximum opening about pH 6.6–6.7, noted previously in coffee leaves, are both found in *Chrysanthemum leucanthemum* and *Beta vulgaris*.

3. In the six species examined, average starch-content varies with these same two factors and in general along lines of negative correlation with the average stomatal aperture.

4. The chief divergences, from strict parallelism in reduced starch-content and degree of stomatal opening, occur where the limitations of

observation become effective at the stage of "full starch" or at the stage of "no starch."

5. Activity of amylase enzyme systems is indicated as the factor, which is, in its turn, controlled by the pH and by the kind of buffer-system used, see Small and Maxwell (1939). This would control the average starch-content along approximately parallel lines such as are here recorded graphically.

6. The natural pH ranges of the stomatal guard-cells of sixteen species in dark and light are recorded:

In dark—13 species down to pH 4.0, 3 species down to pH 4.4;

In light—8 species up to pH 5.2, 4 lower up to pH 4.4–5.0, 3 higher up to pH 5.6, and 1 to pH 6.2.

Coffea arabica leaves, pH 4.0–6.2, show the widest range. The common range, pH 4.0 or 4.4 to pH 5.2, should be noted in conjunction with minimal carbonate and phosphate buffering in this zone (Small, 1929, p. 363) and with the effect of carbon dioxide on sunflower sap, from pH 5.6 to pH 4.5 with 40 per cent. CO₂, and pH 4.0 with 85 per cent. CO₂ (*ibid.*, p. 333).

Part IV. Time Relations of Stomatal Movements.

By Professor J. SMALL, D.Sc., and Miss M. I. CLARKE, M.Sc.

The time of less than two minutes, claimed by Nutman (1937) for his observations of changes in stomatal aperture under all natural conditions of varying illumination, indicates that at least the beginnings of natural stomatal movements are comparatively rapid in coffee leaves.

Under controlled conditions of short alternating periods of light and darkness the time interval for initiation of opening might appear to be reduced, for *Pelargonium*, to less than one minute, with the initiation of closing taking more than one minute, according to Gregory and Pearse (1937), but these short alternations of light and dark periods may give a cumulative reaction and may not be strictly comparable with the effects of continuous exposure to some degree of light or to darkness.

As there are no concomitant observations of pH for the guard-cells in the above records, and few details available of the time relations of stomatal movements with varying pH values, an investigation of these points was undertaken.

The initiation of change and its progression to some degree can be studied by means of Nutman's records of porometer readings over a period of three hours. His chart (1937, fig. 4, p. 684) presents the relation between stomatal aperture and solar radiation. Transferring

this chart, on a larger scale, to graph paper, the times taken for temporary opening and for temporary closing may be measured. The first opening is completed in 22 minutes, and the second in 40 minutes. The first closure takes 23 minutes and the second closure 15 minutes. These data definitely refer to some stages of opening and closing beyond the initial stages, and are mentioned again below.

METHODS.

Stripping.—Strips of epidermis were carefully removed by means of small forceps from the under side of young leaves, preferably from just behind the tip. It was observed by Desai (1937) that this region of the leaf has particularly sensitive stomata.

Washing.—The strips of epidermis were immersed in neutral water (Small, 1929) for not more than ten minutes.

Application of Buffer Solutions.—The pH for the maximum stomatal opening and closing for six of the plants was obtained from figs. 1 to 6 (in Small and Clarke, Part III of this series). Similar data for the other plants were obtained from Small and Maxwell (1939). Corresponding buffer solutions were prepared and used. Where stomatal opening was being investigated, leaves which had been kept over-night in the dark were stripped and washed. One strip was placed on a slide, with a cover-slip, and examined. One drop of buffer solution of the pH required to open the stomata was placed on one side of the cover-slip and drawn through to the strip from the other side by means of filter paper. Where stomatal closing was being investigated, leaves taken from plants under normal light conditions were used.

Examination.—The strips of epidermis were examined as soon as they were placed on the slide, and again immediately after application of the buffer solutions. This second examination was continued during the whole of the opening or closing movement, until such movement was completed.

Timing.—Applications of the buffer solution was used as zero time in all cases. Where stomatal opening was being observed, the time was noted (*a*) when the stomata first showed signs of any definite opening, and (*b*) when they were fully opened. Where stomatal closure was being observed, the time was noted (*a*) when the stomata first showed signs of any definite closing, and (*b*) when they were fully closed.

Since this method of controlling the opening and closing of stomata depends upon the penetration into the guard-cells of the outwardly applied buffer solutions, it is to be expected that the rate of penetration

of the buffer substances, unless it is relatively very rapid, will have some effect on the speeds shown by the stomatal movements.

RESULTS.

The main experimental results, as times in minutes, are presented here in Table I.

A subsidiary point of some interest was investigated in coffee leaves. It was found that, after immersion of epidermal strips for half an hour in buffer solutions, starch appeared to be absent from the guard-cells of the stomata, between pH 5.4 and pH 5.7 in acetate buffers, and between pH 6.5 and 6.9 in phosphate buffers. As these stomata are fully open in these buffers in 70 and 60 minutes respectively, it would appear that the failure of the iodine-starch coloration is followed by the production of osmotically more active non-colouring products during the progressive opening of the stomata, as indicated previously (Small, Part II of this series; cp. Hanes, 1937, pp. 202 and 210).

Opening Times.—The main data for stomatal opening are given in the left half of Table I, with the plants placed in the order of rapidity of opening in citrate buffers. The selection of the citrate series is mostly quite arbitrary, and the shortest time is actually 2.5 minutes for *Rumex acetosa* in phosphate solution.

The time-range for slight opening from fully closed is 2.5 to 20 minutes, while the time-range for full opening from fully closed is 20 to 180 minutes. Stomatal opening to full extent appears to be a leisurely movement, but from a physiological point of view partial opening can be very effective and some degree of opening with at least one of the buffers used is obtained within less than 8 minutes with all the plants used except *Pelargonium* and *Bryophyllum*.

The speed of initiation of opening varies considerably, not only with the pH and with the material, but also for the same material used with different buffer substances. While 14 out of 16 open partially within 8 minutes in at least one buffer solution, only 7 of these 14 open partially within 5 minutes.

There is a variation of 3 to 18 minutes in *Beta* according to the buffer used, but other plants show less variation with the kind of buffer used. Ox-eye daisy (4-7), iris (4-9), sorrel (2.5-6), tulip (7-10), *Inula* (6-9), bean (8-10), and *Bryophyllum* (17-19) are examples of fairly steady times for partial opening in different buffers. Many factors may influence the time observed for the beginning of opening, such as the actual pH within narrow limits, the influence of the kind of buffer on the activity of the

enzyme system, the time required for penetration of the buffers into the guard-cells, the mechanical arrangement and strength of the guard-cell

TABLE I.

PLANTS	OPENING			NATURAL pH RANGE	CLOSING		
	FROM FULLY CLOSED TO SLIGHTLY OPEN MINS.	TO FULLY OPEN MINS.	PH BUFFERS CIT. ACET. CIT. PHOS. BOR.		FROM FULLY OPEN TO HALF OPEN MINS.	TO FULLY CLOSED MINS.	PH BUFFERS CIT. ACET. CIT. PHOS. BOR.
1. <i>TRADESCANTIA</i> <i>zebrina</i>	3 $\frac{7}{4}$ ₃	40 $\frac{60}{30}$ ₂₀	4.2 $\frac{4.7}{8.7}$ _{8.7}	4.0-5.0	3 $\frac{3}{1}$ ₁	7 $\frac{0.5}{14.2}$ _{14.2}	5.6 $\frac{5.7}{9.8}$ _{9.8}
2. <i>RUMEX acetosa</i>	5 $\frac{6.5}{8}$ ₈	30 $\frac{60}{30}$ ₃₀	4.5 $\frac{4.3}{8.7}$ _{8.3}	4.0-4.6	4 $\frac{3}{3}$ ₅	30 $\frac{25}{10}$ ₁₀	6.0 $\frac{5.2}{8.8}$ _{8.8}
3. <i>CHRYSANTHEMUM</i> <i>leucanthemum</i>	7 $\frac{4}{5}$ ₆	60 $\frac{40}{50}$ ₅₀	3.2 $\frac{3.7}{8.9}$ _{8.9}	4.4-5.2	4 $\frac{2}{2}$ ₂	9 $\frac{12}{30}$ ₃₀	5.0 $\frac{4.6}{9.2}$ _{9.2}
4. <i>TULIPA</i> sp.	7 $\frac{8}{70}$ ₇₀	40 $\frac{110}{100}$ ₁₀₀	4.5 $\frac{4.3}{8.6}$ _{8.6}	4.0-5.2	5.5 $\frac{8}{10}$ ₁₀	8 $\frac{20}{38}$ ₃₈	3.4 $\frac{3.6}{8.4}$ _{8.4}
5. <i>IRIS pseudacorus</i>	8 $\frac{8}{49}$ ₄₉	90 $\frac{120}{45}$ ₄₅	4.7 $\frac{4.8}{8.7}$ _{8.3}	4.0-5.2	→ 4 $\frac{6}{3}$ ₃	0.5 $\frac{12}{28}$ ₂₈	2.3 $\frac{3.4}{9.8}$ _{9.8}
6. <i>INULA helenium</i>	9 $\frac{10}{68}$ ₆₈	30 $\frac{120}{50}$ ₄₀	4.6 $\frac{4.3}{8.3}$ _{8.3}	4.0-5.2	1 $\frac{2}{5}$ ₅	2 $\frac{18}{1}$ ₁	5.4 $\frac{5.4}{8.6}$ _{8.6}
7. <i>MARCISSEUS</i> <i>pseudomarcisus</i>	9 $\frac{11}{76}$ ₇₆	110 $\frac{80}{30}$ ₃₀	5.1 $\frac{5.4}{9.2}$ _{9.2}	4.0-5.2	4.5 $\frac{5.4}{4}$ ₄	14 $\frac{19}{30}$ ₃₀	3.8 $\frac{3.4}{4.8}$ _{4.8}
8. <i>VICIA faba</i>	10 $\frac{8}{10}$ ₁₀	100 $\frac{80}{110}$ ₁₁₀	5.1 $\frac{5.0}{8.0}$ _{8.0}	4.4-5.6	2 $\frac{4}{8}$ ₈	4 $\frac{17}{35}$ ₃₅	2.2 $\frac{3.6}{4.8}$ _{4.8}
9. <i>CENTRANTHUS ruber</i>	12 $\frac{6}{75}$ ₇₅	140 $\frac{100}{85}$ ₈₅	6.1 $\frac{5.9}{8.3}$ _{8.2}	4.4-5.2	2 $\frac{4}{5}$ ₄	18 $\frac{13}{72}$ ₇₂	4.8 $\frac{4.4}{8.7}$ _{8.7}
10. <i>HYACINTHUS</i> sp.	12 $\frac{7.5}{11.5}$ _{11.5}	80 $\frac{70}{30}$ ₃₀	4.6 $\frac{5.2}{8.5}$ _{8.2}	4.0-5.2	2 $\frac{1}{7}$ ₇	9 $\frac{12}{20}$ ₂₀	3.0 $\frac{3.2}{8.5}$ _{8.5}
11. <i>PELARGONIUM</i> <i>zonale</i>	12 $\frac{20}{18}$ _{10.5}	150 $\frac{120}{40}$ ₄₀	4.7 $\frac{4.5}{7.7}$ _{8.0}	4.0-5.6	11 $\frac{13}{12}$ ₁₂	15 $\frac{30}{37.4}$ _{37.4}	5.4 $\frac{5.7}{9.5}$ _{9.5}
12. <i>KNIPHOFIA</i> sp.	13 $\frac{7.5}{12}$ ₁₂	150 $\frac{120}{70}$ ₇₀	5.6 $\frac{5.9}{8.8}$ _{8.8}	4.0-5.2	1 $\frac{0.5}{8.5}$ _{8.5}	7 $\frac{14}{13.2}$ _{13.2}	3.9 $\frac{5.0}{8.7}$ _{8.7}
13. <i>RUMEX obtusifolius</i>	15 $\frac{9.5}{14}$ ₁₄	120 $\frac{130}{30}$ ₃₀	5.6 $\frac{4.8}{8.6}$ _{8.6}	4.0-5.6	1 $\frac{5}{3.5}$ _{3.5}	3 $\frac{11}{38}$ ₃₈	2.0 $\frac{3.6}{8.8}$ _{8.8}
14. <i>COFFEA arabica</i>	16 $\frac{5.5}{15}$ ₁₅	180 $\frac{70}{80}$ ₈₀	5.6 $\frac{5.7}{8.8}$ _{8.8}	4.0-6.2	1 $\frac{2.5}{4}$ ₄	2 $\frac{20}{43}$ ₄₃	1.2 $\frac{3.3}{9.0}$ _{9.0}
15. <i>BRYOPHYLLUM</i> <i>calycinum</i>	17 $\frac{19}{18}$ ₁₈	70 $\frac{90}{180}$ ₁₈₀	5.0 $\frac{4.8}{8.8}$ _{8.8}	4.0-4.4	3 $\frac{6}{8}$ ₈	7 $\frac{21}{44}$ ₄₄	2.2 $\frac{4.3}{5.4}$ _{5.4}
16. <i>BETA maritima</i>	18 $\frac{4.5}{7}$ ₇	60 $\frac{40}{30}$ ₃₀	5.6 $\frac{5.7}{8.7}$ _{9.0}	4.0-4.4	3 $\frac{9}{1.5}$ _{1.5}	10 $\frac{0.5}{25}$ ₂₅	3.0 $\frac{4.8}{5.7}$ _{10.0}
TIME RANGE (MINS)	2.5-20	20-180			→ 7-13	0.5-50	∞
→ = LESS THAN 30 SECONDS.							

walls and so on. Therefore no detailed analysis of these time-relations is likely to be profitable at this stage.

The general phenomena are quite obvious. (a) The time for partial opening varies from 2.5 to 20 minutes, and is commonly 8 minutes or less in at least one of the buffers used. (b) The time for full opening varies from 20 to 180 minutes and is commonly about one hour, frequently more, sometimes less.

Closing Times.—The main data for stomatal closing are presented in the right half of Table I, following the order established for opening as a matter of convenience.

The time-range for partial closing from fully open is less than 30 seconds (\rightarrow) up to 13 minutes, while the time-range for complete closure from fully open is half a minute up to 50 minutes. Complete stomatal closure appears to be relatively rapid, when compared with complete opening. In all cases, except *Pelargonium* and *Narcissus*, there is partial closure in 3 minutes or less in at least one of the buffer solutions used.

The speed of the initiation of closing varies according to the pH, the material, and the kind of buffer used. This variation is great in beet (less than 0.5 up to 9 min.), in tulip (3–10 min.), and in *Kniphofia* (0.5–8 min.); but *Tradescantia* (0.5–3),* coffee (0.5–4), and *Inula* (0.5–5) can be described as usually rapid; ox-eye daisy (2–4), sorrel (3–5), *Centranthus* (2–5), and *Narcissus* (4.5–5) as consistently of medium speed for partial closure, and *Pelargonium* (8–13) as consistently relatively slow to show half-closure. *Iris* varies from less than 0.5 up to 6 minutes, dock and hyacinth from 1 to 7 minutes, bean from 2 to 8 minutes, and *Bryophyllum* from 3 to 8 minutes for partial closure.

Various factors may influence the speed of closure, including most of those specified for opening, but whereas the mechanical structure of the guard-cells tends to oppose opening, it would tend to assist closing, and this may be one of the factors which produce the observed differences between relatively slow complete opening and relatively not so slow complete closure.

Speeds within the Natural Range of pH (Table I, heavy figures).

These experimental results may remain as isolated observations, of interest only to specialists, unless they can be connected up with physiological reactions under natural conditions.

The highest speed for partial opening (2.5 min.) was obtained with sorrel in phosphate at pH 6.7, but within the natural range of pH for the guard-cells of that leaf (pH 4.0–4.6) the speed was 5 to 6 minutes in citrate (pH 4.5) and acetate (pH 4.3). The highest speed for partial opening in *Tradescantia* (3 min.) was obtained within its natural range (pH 4.0–5.0) in citrate, but in acetate at pH 4.7 the time increased to 7 minutes. These are examples of the kind of variation shown. Including only the times within the natural range of pH for the guard-cells, ten of the sixteen plants show speeds of 3 to 9 minutes for partial opening,

* Scarth (1926, p. 1156) records, for *Zebrina*, with graphs but no exact figures, "rapid changes of posture (notable within less than half a minute) follow changes in pH."

while four of the other plants show similar times outside their natural range. The other two are *Bryophyllum* (17 to 19 min.) and *Pelargonium* (12.5 to 20 min. within its natural range of pH).

For partial closure many of the higher speeds obtained were outside the natural range of pH, but there is one example, *Kniphofia*, showing 0.5 and 1.0 minute within its natural range. The other heavy type values vary from 2 to 10 minutes with the exception of *Pelargonium* which is again relatively slow (11 to 13 min. within its natural range). Since it was in *Pelargonium* that Gregory and Pearse (1937) found a minimum opening with 45 seconds periods of alternations, it may be pointed out here—(a) that Scarth (1926) without giving definite times stated that he found *Pelargonium* slower than *Tradescantia*, and (b) that our *Pelargonium* showed slightly slower opening than closing, as compared with the quicker opening than closing found by Gregory and Pearse. The difference between speeds for partial opening and partial closing in *Pelargonium* are, however, less than in many other plants; for *Pelargonium* in the same buffers partial opening and partial closing show 12.5–11, 20–13, 18–12, 10.5–8. For *Tradescantia* similar pairs (opening–closing) are 3–3, 7→, 14–0.5, 3–1.

Comparing the highest speeds in the natural pH range, for partial opening and partial closing, we get: 3, 5, 5, 7, 7.5, 8, 8, 9, 9, 9.5 (12.5) for opening, and for closing (0.5), 2, 2.5, 4, 4.5, 6, 6, 7, 8, 10, (11). Although the general tendency seems to be for a more frequent higher speed for closing than for opening, the difference between a range of 2 to 8 or 10 and a range of 3 to 9 does not seem to be really significant. Since these partial changes of aperture are highly significant in relation to gaseous diffusion and to stomatal functions, and since they would appear to take place with approximately very nearly the same speed at natural pH values, our final conclusion must be in this sense.

So far as normal functioning of the stomata is concerned the effective but partial opening and closing of the stomata, under our conditions, takes place at about the same speed, in a normal range of 3 to 10 minutes. From these time intervals some proportion may be deducted for the penetration of the buffer substances. This proportion may be high or low according to the rate of penetration for any given plant and any of the four buffers.

Quantitative data for the rate of penetration in very short periods of time are only beginning to appear, but some idea of the position may be given by quoting Brooks (1939, p. 387) who writes: "The most impressive result of these experiments is the almost instantaneous entry of the radioactive isotope into the protoplasm of these living cells."

Solutions of K^*Cl were used with *Nitella*. The meaning to be attached to "instantaneous" may be gathered from a continuation of the quotation: "This is shown particularly well by an experiment shown in fig. 1. At three concentrations, 0.02, 0.01, and 0.005 M, of the K^*Cl solution as the immersion fluid, the radioactive ion content of the cell protoplasm markedly exceeds the external concentration after 5 minutes, or even after only 1 minute's immersion in a 0.02 M solution." Again (Brooks, 1940, p. 172): "In the same way both K and Na enter *Spirogyra* cells and reach concentrations exceeding ten-fold that of the immersion solution within 15 seconds."

Our observations show that complete closure is more rapid than complete opening, even within the natural range of pH for the guard-cells. The highest opening speeds can be arranged thus: 30, 30, 40, 40, 70, 70, 80, 80, 90, 120, 120; while the highest closing speeds can be read as 7, 9, 13, 15, 20, 21, 23, 25, 35, 40, 50. The mechanical structure of the guard-cells, opposing opening and tending towards closure, is a possible explanation of the obvious difference which may be connected with the functioning of stomata under natural conditions. Whereas the time-period for complete opening is frequently about ten times that for partial opening, the time-period for complete closure is frequently only three or four times that for partial closure.

Along these lines, the above-mentioned data derived from Nutman's chart may be explained. The first opening took 22 minutes and the first closure 23 minutes; these were possibly a partial opening and partial closure of stomata at equal speeds; the second opening took 40 minutes and the second closure 15 minutes; this was possibly a more complete opening followed by some degree of closure at higher speed.

SUMMARY.

1. In the sixteen species examined, the time taken for stomatal movements, controlled to definite pH values by external application of buffer solutions, varied within limits according to (a) the kind of buffer used and (b) the material used.

2. The results which are of significance for normal physiological functions, as obtained within the natural range of pH for the guard-cells, may be grouped thus:

usual times for partial opening—3 to 9 min.,
complete opening—30 to 90 min.,
partial closing—2 to 8 min.,
complete closing—7 to 40 min.

The exceptions to this general statement will be found in Table I. Natural movements, controlled by light and internal changes of pH, possibly take place with higher speeds, since they do not involve the penetration of outwardly applied buffer substances.

3. The time-periods for complete opening are frequently ten times those for partial opening, while the time-periods for complete closure are frequently only three or four times those for partial closure.

4. In all cases these time-periods were determined at or about the pH where maximum opening or maximum closing had been found in previous investigations. These speeds, therefore, are probably optimal values for our conditions of external control. The *degree* of opening or closing definitely varies within narrow ranges of pH. The *speed* of movement may vary in the same sense.

Part V. Time and pH Relations in Amylase Activity.

By Professor J. SMALL, D.Sc., and Mrs J. CROSBIE-BAIRD.

Several botanical writers have suggested that enzymic activity is too slow to account for the relatively rapid movements of stomatal guard-cells. The optimal pH values for stomatal opening occur at or around some of the many ranges for optimal activity of amylases from different sources, and also vary with the buffers used (Appendix I). Enzymic action by a variety of amylases or by the same amylase under different conditions is indicated.

Rapid action of salivary amylase on a relatively small quantity of weak starch solution can be readily and simply demonstrated using saliva, followed by the iodine test, and a control.

Various quantitative investigations have been made involving the velocity of amylase action down to initial periods of 42 seconds and one to ten minutes (Hanes, 1932, and Tauber, 1937). Apparently, however, there are no records of reaction velocity which deal simultaneously with *short* periods of enzymic action and with pH variations.* Since explanations of stomatal movements must cover effective action in 30–120 seconds, and since such action is apparently controlled by slight pH variations, the available data required extension to meet this special case.

The first clue to the technique used was obtained in 1939 when Crosbie as a student used an old sample of malt extract in a class experiment with 0.1 per cent. starch solution and reported that all the starch dis-

* Hanes, 1932, in a footnote promised a discussion of pH-activity relationships in a subsequent paper, but that has not been traced.

appeared in less than three minutes, blue with iodine re-appearing again later. This led to investigations which are here summarised briefly.

VELOCITY OF AMYLASE ACTION BY THE IODINE METHOD.

Buffer Solutions.—Sørensen's phosphate mixtures were used to make solutions of pH 4.7, 5.8, 6.4, 6.6, 6.8, 7.0 and 8.0 (Clark, 1928). Walpole's acetate mixtures were used to make solutions of pH 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.5, 5.6, 5.8, 6.0, 6.2 and 6.8 (Walpole, 1914).

Amylase Solutions.—Two types were used, prepared freshly each day; 1—malt extract, 6 g. dissolved in 100 c.c. buffer solution or distilled water; 2—diastase (B.D.H.), 1 g. in 500 c.c. buffer solution or distilled water.

Starch Solution.—Pure potato starch, 1 g. shaken with 100 c.c. water and poured into 900 c.c. water; a few drops of toluene added as a preservative.

Iodine Solution.—Iodine 10 g., with potassium iodide 5 g., dissolved in 1000 c.c. distilled water.

Amounts of Solutions.—A set of six test-tubes was used for each experiment. Into each tube 5 c.c. of starch solution and 5 c.c. of amylase solution were pipetted and mixed. After specified time-intervals 5 drops of iodine solution were added to each tube and the resulting colours noted until the achroic point was reached.

RESULTS.

1. Malt Extract: (a) with distilled water, no buffer, pH 5.6, temperature 63° F. An average of ten tests gave 2.5 minutes for failure of the blue coloration with iodine.

(b) With phosphate buffer, minimum times for the disappearance of *all* blue colour were 22 minutes at pH 6.4, 4.5 minutes at pH 6.6, 5.5 minutes at pH 6.8.

(c) With acetate buffer, minimum times for the disappearance of *all* blue colour were 19 minutes at pH 5.4, 16 minutes at pH 5.6, 12.5 minutes at pH 5.8.

Distilled water at pH 5.6 thus appears to give more rapid enzymic action than either of the buffers used, but these malt extracts results are complicated by the occurrence of a series of colour changes from the typical blue black of starch-iodide through brown, green, greenish-blue, clear blue to a final yellow-orange tint. The changes from brown through greens to pure blue took place in phosphate slowly at pH 6.4 (3 to 28 min.), more rapidly at pH 6.6–6.8 (< 0.5 to 4 min.); in acetate

slowly at pH 5.4 (1 to 14 min.), more rapidly at pH 5.6 (0.5 to 2 min.) and at pH 5.8 (usually 1 min.). The subsequent change from pure blue to yellowish took longer as a rule, as is indicated by the data for the disappearance of *all* blue colour.

These changes were of interest because the slow sequences, at pH 6.4 and 5.4, clearly indicated progressive reaction with possible formation of a product giving another kind of blue colour with iodine. The colour series occurred only in the presence of buffer-salts and iodine, not in distilled water and iodine, and it was very slow only at pH 6.4 in phosphate when the iodine was added at intervals of 5 minutes; the second blue disappearing in 11 to 28 minutes. Some effect may be attributed to the iodine which reduces the activity of malt amylase 48 per cent. in 10 minutes at an iodine concentration of 1.39×10^{-7} N (Waksman, 1926, p. 172). The iodine as present in these experimental mixtures was about .002 N. The phosphate and acetate are both possible activators of amylase action, acting in opposition to the iodine retardation to a differential degree on the two or more amylases present in malt extract, see below in the Discussion.

An early experiment with a particular sample of malt extract, in acetate buffer at 65° F., showed a definite optimum at pH 5.5 for the failure of blue with iodine and starch, thus:

pH	5.8	5.6	5.5	5.4	5.2
min.	205	115	55	80	200

2. Diastase (B.D.H.): (a) With distilled water, no buffer, pH 5.6, temperature 60° F. Failure of the blue coloration with iodine occurred in 3 minutes.

(b) With phosphate buffer, pH 4.7–8.0; reddish brown replaced the blue of starch iodide in minutes at various pH values thus:

pH	4.7	5.9	6.4	6.6	6.8	7.0	8.0
min.	2	3	4	2	2.5	4	9

Minimum time for failure of blue coloration is two minutes.

(c) With acetate buffer, pH 4.0–6.8; red colour replaced the blue of starch iodide in minutes at various pH values thus:

pH	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.8
min.	18	21.5	4	3.75	4	3.5	5	3	2.5	2.75	4	18.5	5.75

Minimum time for the failure of blue is 2.5 minutes at pH 5.6.

These results are graphed in fig. 1, and show optima for amylase activity as reaction velocity; in phosphate possibly at pH 4.7 and at pH 6.6, but there was only slight variation in the range used; in acetate

from pH 4.4 to 6.0 with slight variations within that range showing a definite optimum at pH 5.6, and signs of a possible second optimum

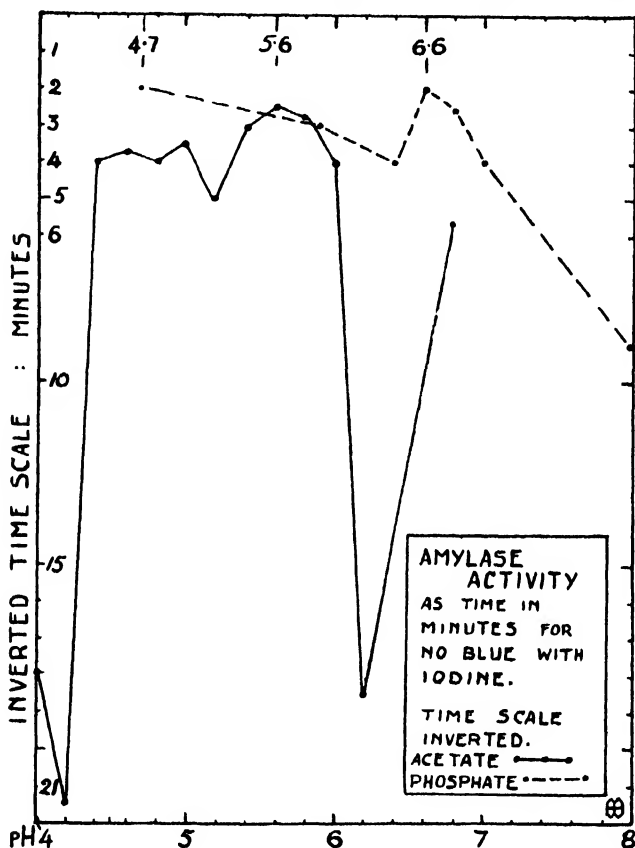


FIG. 1.

about or above pH 6.8. The time-scale in fig. 1 is inverted so that enzymic activity can be read in the usual fashion.

VELOCITY OF AMYLASE ACTION BY REDUCTION METHOD.

Buffer Solutions.—Walpole's acetate buffer solutions were used as before, pH 4.0–6.4 in steps of 0.2 pH unit.

Amylase Solution.—B.D.H. diastase containing some maltose was used, 1 g. in 500 c.c. of buffer solution.

Starch Solution.—0.1 per cent. potato starch prepared as before.

Methylene Blue Solution.—1 g. of methylene blue dissolved and made up to 100 c.c. in distilled water.

Fehling's Solution.—Prepared and diluted, 10 c.c. Fehling solution diluted to 100 c.c. with distilled water.

Method.—100 c.c. amylase solution (in buffer) were placed in a 500 c.c. beaker with 100 c.c. starch solution and stirred at intervals. After given intervals of time (2, 3, 5 min.) for enzymic action the mixture was boiled for 1 minute, allowed to cool, and filtered twice through double thickness of filter paper to remove the agglutinated protein.

25 c.c. of diluted Fehling solution were titrated with the filtrate, using a first addition of 15 c.c. filtrate and bringing the mixture to the boiling-point. Further portions of the filtrate were added until only a faint blue remained. Three drops of 1 per cent. methylene blue were then added and the addition of filtrate continued drop by drop until the dye was completely reduced and no blue colour remained. Three titrations were made for each pH value, and these were found to be reasonably consistent, the end-point being easy to observe.

Maltose in the Diastase.—Using the above titration method with the diastase in distilled water and no starch, it was found that the diastase was loaded with maltose to such an extent that 1 g. of the "material" contained 0.2356 g. of maltose, so that each 100 c.c. of 0.2 per cent. solution contained 47.13 mg. maltose. This was taken as a constant and deducted from all the quantities subsequently determined. Although part of the reduction may be due to dextrans (Hanes, 1937), the reducing substances were calculated as maltose, following the usual practice. The final results were calculated to a basis of the action of 2 grams of "diastase" material on 1 gram of starch mixed in two litres of water. In the digest the concentration of diastase material was 0.1 per cent. and of starch 0.05 per cent. These data are useful for comparisons made in the discussion which follows. The results are presented as Table I and fig. 2.

From Table I and fig. 2 we can at once select the following points:—

1. Optimal pH value is pH 5.6 in acetate buffer for this amylase preparation; the same as was found with the iodine method using the same buffer.

2. Measurable quantities of reducing substance can be produced by enzymic action on starch in very short periods of time; extending in two minutes at optimal pH to 61 per cent. conversion of the starch.

3. The *differences* in hydrolysis at various pH values are significantly large. Those between pH 4.4 and pH 5.6, as percentage differences on the lower values, are at two minutes 21 per cent., three minutes 48 per cent., five minutes 28 per cent.

4. The differences in hydrolysis are larger, per change of 0.2 pH unit, on the alkaline side of the optimal pH than on the acid side. This is

TABLE I.—MG. MALTOSE PRODUCED BY THE ACTION OF 2 G. B.D.H. DIASTASE ON 1 G. STARCH AT 18.5° C. IN TWO LITRES OF WATER.

pH	In 2 min.	In 3 min.	In 5 min.	Per cent. Conversion in 2 min.
4.0	..	344.9	498.0	..
4.2	..	463.2	564.5	..
4.4	503.0	484.3	620.9	50.3
4.6	507.0	526.2	644.5	50.7
4.8	520.4	565.3	664.2	52.0
5.0	559.1	581.8	669.2	55.9
5.2	586.4	612.5	691.5	58.6
5.4	595.9	658.9	762.4	59.6
5.6	610.5	718.7	792.9	61.0
5.8	484.5	540.3	611.8	48.4
6.0	433.6	480.7	547.9	43.3
6.2	400.2	462.6	524.4	40.0
6.4	337.2	426.8	499.2	33.7

very obvious on inspection of the column in Table I showing percentage conversion.

These points clearly demonstrate the possibility of rapid and effectively large changes in enzymic activity with changes of pH values in

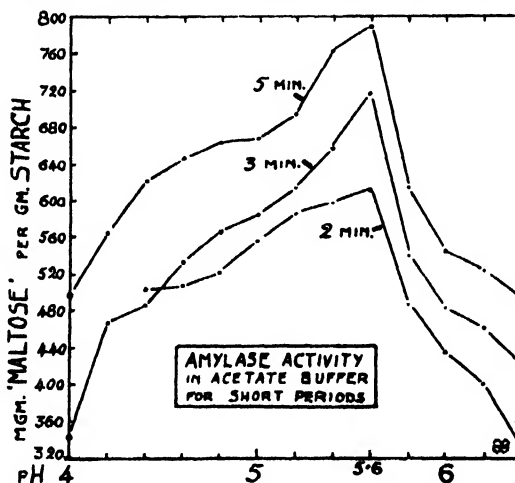


FIG. 2.

stomatal guard-cells, such changes being within the natural range of stomatal pH found for coffee leaves (pH 4.4–6.2), and near the common range of pH 4.0–5.2.

DISCUSSION.

The solution of the main problems concerning the mechanism of stomatal movements is completed by the data presented above. The short periods of time, 2–3 minutes, observed for stomatal movements can be explained by enzymic action which is here shown to be sufficiently rapid to be effective in the available time, and also sufficiently affected by slight changes in pH values to be altered effectively in quantity by the changes in natural pH values observed in stomatal guard-cells.

The new data, however, are of some importance in the general interpretation of the relation of pH values to enzyme activity, and a discussion of that significance seems to be required since it has not been seen in any of the standard works on enzymes.

First of all it is necessary to establish the validity of the data by comparison as far as possible with other records. The large percentage conversion of starch in a few minutes is one point upon which perfectly reasonable suspicion might be cast, but comparison with previous results, recorded by Hanes (1932) and others, show that these percentages are at least known to biochemists if still comparatively unappreciated by some botanists.

Hanes (1932, p. 1413) records results with a particular amylase preparation (opt. pH 4.6–4.8) in acetate buffer with a final pH 4.71–4.73, using various percentage concentrations of starch from 1.078 to 0.005. Selecting some of these, especially the 0.05 per cent. which corresponds to the strength of the starch solution used here, and extending Hanes' data in a very plain calculation of percentage conversion, we obtain Table II.

As presented here the percentage conversions, calculated from Hanes' data on the left, can be easily checked, and there seems no doubt about conversions up to 17.6 per cent. in 2 minutes with 0.05 per cent. starch, and up to 40 per cent. in 2 minutes with 0.005 per cent. starch. Hanes' record of 72 per cent. conversion in 8 minutes with 0.005 per cent. starch compares with our highest conversion, 79.3 per cent. in 5 minutes with 0.05 per cent. starch. The differences may be due to variations from the optimal pH value or to the difference in the enzyme used. Since reaction velocity varies with the concentration of the enzymes, either directly or logarithmically or with some intermediate relation, and the two sets of results are being compared without reference to their relative concentrations or differing sources, the general lines of agreement are quite reasonable. Hanes obtained 49.6 per cent. conversion in 10.1 minutes and Crosbie-Baird obtained 61 per cent. conversion in 2 minutes using the

TABLE II.—SELECTED FROM HANES' TABLE II, 1932, AND EXTENDED ON THE RIGHT.

Starch, per cent.	Digestion in min.	Maltose,		Starch, g. in 100 c.c.	Per cent. Conversion of Starch.
		mg. in 5 c.c.	g. in 100 c.c.		
·050	0·9	·23	·0046	·0500	9·2
	2·1	·44	·0088		17·6
	10·1	1·24	·0248		49·6
·040	0·7	·155	·0031	·0400	7·75
	1·9	·34	·0068		17·0
	3·0	·50	·0100		25·0
·005	0·7	·06	·0012	·0050	24·0
	2·0	·10	·0020		40·0
	8·0	·18	·0036		72·0
·216	0·7	·415	·0083	·216	4·1
	2·3	·95	·0190		8·8
Hanes' data				extension	

same concentration of starch but two quite different amylase preparations. The validity of the new data would appear to be established as to quantitative accuracy *at optimal pH value*. It seems logical, therefore, to consider that the methods used and the results obtained have equal validity *over the range of pH values used*; and this is a critical aspect in relation to their application (*a*) to stomatal movements and (*b*) to general conceptions.

The relation of pH to enzymic action may involve some degree of control over the reaction velocity or over the final state of equilibrium or over both.

The view that amylase has only a one-way action, hydrolysis, at optimal pH is supported by the 100 per cent. yield of maltose from 2 per cent. starch by α -amylase at pH 4·5 in ·01 M acetate at 40° C. after 1300 minutes (Sherman *et al.*, Tauber, 1937, p. 144). During this digestion 31 per cent. conversion occurred in 30 minutes, with an even rise, more or less proportional, and *red* colour with iodine was given at that point. With β -amylase the conversion curve was different, going to achroic with iodine at 2700 minutes and showing a much higher initial rate, about 50 per cent. in 30 minutes and about 25 per cent. in 10 minutes, but blue with iodine persisted for many hours in this case. The higher concentration of starch, 2 per cent., lowers the reaction velocity (compare Hanes' data in Table II), but the main point to be noted here is that

under specified conditions full hydrolysis is obtained and there is no evidence for concomitant synthesis under these conditions. The control exerted by the pH in this case must be taken as over the reaction velocity only.

If the control by pH is to be considered as over both reaction velocity and final equilibrium, we must first establish some possibility or probability of synthesis as well as hydrolysis of starch. Again Hanes has furnished substantial evidence and may be quoted thus: "Of considerable botanical interest is the fact that the conversion of starch to glucose-1-phosphate is a reversible reaction: this is catalysed in both directions by an enzyme which will be referred to as a phosphorylase" (1940 *a*). The synthesis of starch is an accomplished fact, even to the stage of solid grains (Hanes, 1940 *b*). This occurs in the presence of a phosphate and an enzyme which also breaks down starch and which, therefore, may be classed with the other amylases, although it is quite reasonably distinguished as having a particular type of action, esterifying the glucose by the addition of a phosphate radicle and therefore a phosphorylase. On the subject of the pH relations of this reaction, which is a preliminary in starch synthesis, Hanes is quite precise (1940 *a*): "The esterification of phosphorus was found to be strongly affected by pH changes between pH 4.9-5.7 and from pH 7 to pH 8, with a maximum between pH 5.7 and pH 7." The pH curve for glucose-1-phosphate given by Hanes (1940 *a*, p. 436) shows quite a close approach to the rectangular graph which might result from pH governing only the velocity of a one-way reaction, and the time-period taken was 30 minutes. There was an 800 per cent. increase in synthesis between pH 4.9 and pH 5.7, and about a 700 per cent. decrease between pH 7 and about pH 8.3.

Although this tends to support the view that pH controls only reaction velocity and not the final equilibrium, Hanes is again definite. When starch is synthesised from glucose-1-phosphate *free* phosphate is produced and the ratio of free phosphate to glucose-1-phosphate reaches an equilibrium at 83 : 17 at pH 6.4, and 90 : 10 at pH 5.4. "The ratio . . . attains a value which varies with the concentration of hydrogen ions" (1940 *b*). Hydrolysis of starch by phosphorylase was very active at pH 7.5, more or less complete in 360 minutes without phosphate and in 195 minutes with phosphate. Another point of interest in connection with stomatal movements is that phosphorylase has been found in many plants, including *leaves*, also roots and tubers, particularly potato.

Thus the amylase synthesis of starch which has been implicit in the pH control of stomatal movements is definitely proved in the case of a particular type of amylase, called phosphorylase. This is an enzyme

which does what is required, which is controlled by pH and by phosphate in its reaction velocity and in the final equilibrium; and phosphorylase is also known to occur in leaves.

A demonstration of the presence of phosphorylase actually in the guard-cells is now all that is required to complete the evidence, and there is a high degree of probability in the suggestion that action by phosphorylase or a similar enzyme is seen in many observations of the formation of starch in stomatal guard-cells. This probability is supported by some observations made by Miss M. I. Clarke. She found that the addition of iodine to the mounting medium had the effect of closing the stomata rapidly, and in some cases *producing* starch in the guard-cells. This made rapid observation and averaging of results necessary in Part III of this series. It might appear from this that the iodine solution has the effect of *causing* the sugars present in the guard-cells to be built up into starch. Hanes (1940 *a*, p. 447) records a similar development of starch, giving green then blue with iodine *in vitro* when pre-treatment of pea-extract with a low concentration of iodine inhibited the amylase and left the phosphorylase still feebly active, able to synthesise starch.

Hanes (1940 *a*, 1940 *b*) has clearly furnished evidence which substantiates the general explanation of pH control of enzymic activity in stomatal movements such as has been suggested by many workers and demonstrated in some detail by Small and Maxwell (1939) and in this series II-V.

So far as any amylase or similar enzyme is concerned, only one pH optimum for each has been demonstrated, while for stomatal opening sometimes one optimal peak has been found and sometimes one main peak with one or more subsidiary peaks (Parts I and III of this series). An explanation of these multiple peaks or "optima" is possibly to be found in the complex enzyme systems which may be present in stomatal guard-cells, or in the effects of buffers or other salts; compare the one peak in acetate and two peaks at least in citrate for *Pelargonium zonale*, also similar variation with phosphate and acetate for *Inula helenium* in Part III of this series.

SUMMARY.

1. By the iodine-blue method, "achroic" hydrolysis of 0.05 per cent. starch is found to occur:

- (a) with malt extract in distilled water at pH 5.6 in 2.5 minutes,
in phosphate buffer at pH 6.6 in 4.5 minutes, and
in acetate buffer at pH 5.8 in 12.5 minutes;

- (b) with B.D.H. diastase in distilled water at pH 5·6 in 3 minutes, in phosphate buffer at pH 4·7 and at pH 6·6 in 2 minutes, in acetate buffer at pH 5·6 in 2·5 minutes.

2. By quantitative estimation of increased reducing power in 2, 3, and 5 minutes, it is found that conversion of 0·05 per cent. starch with B.D.H. diastase in acetate buffer at pH 5·6 reaches 61 per cent. in 2 minutes, 72 per cent. in 3 minutes, and 79 per cent. in 5 minutes, with lesser percentage conversions at pH values above and below pH 5·6, between 4·0 and 6·4.

3. A slow return of starch-iodine-blue with malt extract, starch, and iodine in phosphate buffer at pH 6·4 is recorded.

4. These data are discussed in connection with Hanes' percentage conversions in short periods and his synthesis of starch by means of phosphorylase, and it is concluded that effective enzymic action in stomatal movements is probable, and certainly is possible within the various known limits such as a time period of two minutes or less, also both hydrolysis and synthesis being affected by pH variations of small extent and over various ranges.

5. These data complete the evidence required for the general scheme for control of stomatal movement given in Part II of this series.

APPENDIX I.

Optimal pH values for Amylases.

Source.	Optimal pH.	Notes.
Five Phanerogam leaves	5·0-5·4	Sjöberg, 1922 (H)
<i>Phaseolus</i>	5·0-5·5	saccharogenesis (W)
	4·0-6·0	liquefaction (W)
<i>Aspergillus oryzae</i>	5·3-5·5	in 0·01 M. acetate (T)
	4·8	saccharogenesis (W)
	4·0	in buffer solution (W)
	3·0	liquefaction (W)
<i>Aspergillus niger</i>	3·5-5·5	
potato	6·0-6·5	James and Cattle, 1933
yellow turnip	6·0-7·0	
white turnip, cabbage, carrot,		
<i>Fusarium, Colletotrichum</i>	6·0	(W)
malt.	4·3-4·6	in 0·01 M. acetate at 40° C. (T)
	4·4-4·5	used in industry (Radley)
	4·3	at 25° C. (W)
	6·0	at 69° C. (W)
malt amylase	5·2	Myrbäck, 1926 (H)
	4·6-4·8	Hanes, 1932

Source.	Optimal pH.	Notes.
malt extract . . .	5.6 5.5 or 5.8 6.6	in distilled water or in acetate buffer, Small and Baird in phosphate buffer, Small and Baird
diastase B.D.H. . .	5.6	in distilled water or acetate buffer, Small and Baird
saliva amylase . . .	4.7 or 6.6 5.6 6.6 6.1-6.2 6.9	in phosphate buffer, Small and Baird in acetate (W) in phosphate (W) in phosphate and acetate (W) in chloride and nitrate (W)
pancreatic amylase . . .	6.5-7.1 7.0-7.2 7.1 6.6-7.2 6.5-7.1 6.5-6.8 6.3-6.8	in NaCl .0005-.10 M. (T) in KCl .005-.05 M. in NaBr .01-.20 M. in NaNO ₃ .005-.20 M. in NaClO ₃ .005-.20 M. in NaCSN .05-.20 M. in NaF .10-.30 M.
		(Sherman <i>et al.</i> , ex Tauber) saliva amylase curves are identical with pancreatic amylase in phosphate, chloride, chlorate, and nitrate, Myr- bäck (T)
synthesis of—		
glucose-1-phosphate (pea)	5.7-7.0	Hanes, 1940 <i>a</i>
starch (potato) . . .	5.4-6.4	Hanes, 1940 <i>b</i>

Extracted from Waksman (W), Haldane (H), Tauber (T), and others.

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XX.—On the Feeding and Breeding of *Calanus finmarchicus* under Laboratory Conditions. By **J. E. G. Rayment**, B.Sc., A.M., and **F. Gross**, Dr.phil., Department of Zoology, University of Edinburgh. *Communicated by* Professor **JAMES RITCHIE**, M.A., D.Sc. (With Two Text-figures.)

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INTRODUCTION.

NUMEROUS investigations have already been made on the biology of *Calanus finmarchicus*. The main body of data, however, has been derived from field observations, and while many and important conclusions have been drawn from such work, confirmation of some of them is required by direct experimental methods. Accurate studies on the feeding, metabolism, and breeding of *Calanus* can only be made on living animals in the laboratory. Some such investigations have already been made by Crawshaw, Clarke, Fuller, Marshall, Nicholls and Orr, Harvey and others, but the results have sometimes been obscure owing to the difficulties encountered in keeping *Calanus* in really healthy condition.

It therefore appeared desirable, first, to attempt to maintain, and if possible, to rear *Calanus* under controlled laboratory conditions, and then to use such healthy animals for experimental work.

Only preliminary experiments have so far been carried out, but the results have shown that *Calanus* can certainly be maintained for considerable periods in a healthy condition. Some data on feeding and breeding rates have also been accumulated. In view of present conditions and the possibility of the work being interrupted, it seemed desirable to report these findings, but it is hoped that the work may be continued, and may include extensive experiments, in particular on behaviour and metabolism.

MATERIAL AND METHODS.

Healthy *Calanus* were obtained from plankton hauls, diluted and dispatched in open breffits of sea-water from Millport. Upon arrival at the laboratory the catch was further diluted and placed in a refrigerator, the upper temperature limit of which could be fixed at any point between

5° C. and 12° C. Throughout most of the investigation no heating unit was attached to the refrigerator, and therefore during very cold weather, when the room temperature fell below that of the refrigerator, the temperature inside the experimental chamber fell also. This drop, however, was very rarely more than 2–3° C.

The catch was sorted under a binocular microscope, and adult male and female and Stage V copepodite *Calanus* were identified, and then transferred to crystallising dishes of cooled sterile sea-water by means of wide-mouthed pipettes. After being washed in this medium for at least an hour, the animals were transferred to covered dishes of cooled "Erdschreiber" medium (*cf.* Gross, 1937). All cultures were kept in the refrigerator, which received some light through a window in one wall. Only healthy and active individuals were used.

In many experiments 15 to 25 individuals were placed in about 200 c.c. of medium, but later on only a single male and female were set up in approximately 80 c.c. The animals were fed with pure cultures of diatoms (*Skeletonema*, *Ditylum*, *Chaetoceros*, etc.) and autotrophic nanoplankton flagellates, from cultures originated by Gross at Plymouth (Gross, 1937). Except in experiments specifically designed to investigate feeding rates, food was added in unmeasured quantities (5–10 c.c.), the concentrations obtained being always considerably higher than that found in nature.

The cultures were frequently examined under the binocular microscope; any eggs present were always found on the bottom of the culture dish. They were counted and then transferred by fine-mouthed pipettes to covered glass dishes of 50 c.c. capacity containing cooled Erdschreiber medium. The cultures were changed when the number of faecal pellets became too high, and also when there was any sign of bacterial growth.

RESULTS.

I. *The Survival of Adult Calanus.*

Calanus survived very well at all seasons under the culture conditions, and were always actively swimming except during actual moults. The maximum number of adult animals which could live successfully in 200 c.c. Erdschreiber medium, with excess of food and weekly change of medium, was approximately 20. No significant difference in survival was observable at any of the temperatures employed for culture (5–12° C.), and the copepods also tolerated changes in temperature of at least 5° C., provided that the change was very gradual. Only a very few hardy individuals, however, would withstand a sudden temperature change,

such as occurred once with certain cultures owing to a fault in the cooling system. Thus of a total of 69 females, healthy and active on 19/6/40, only 38 were alive on 20/6/40, and only 25 survived till 23rd June.

Calanus survived successfully on each of the following food cultures, which were those most frequently used (Table I): *Nitzschia closterium* var. *minutissima* (Dr Allen's Plymouth strain), *Chaetoceros pseudocrinitus*, *Skeletonema costatum*, *Ditylum Brightwelli*, *Chlamydomonas* sp. (length 6–8.4 μ ; diameter 9.5–13.8 μ) (cf. Marshall, 1924). They could also utilise the small flagellates D, B.II, F and H, but survival appeared to be less satisfactory when *Calanus* were fed on a flagellate culture only. This was probably due to the fact that the copepods could not filter sufficient food if flagellates, and none of the larger diatoms, were present, unless the cultures were very dense. Masses of faecal pellets resulted from a day's feeding on any of the diatom or flagellate foods, and a dense culture would often be swept almost clean. Flagellate D is yellowish-green, 1.3–2.0 μ long (average 1.7 μ); 1.0–1.3 μ wide (average 1.2 μ). B.II is a Chrysomonad with golden-brown pigment; length 3.8–5.2 μ (average 4.6 μ); width 3.0–4.2 μ (average 3.7 μ); two flagella, 4.6–7 μ long. Flagellate F is faintly green and opaque, 1.6–2.7 μ long (average 2 μ); width 1.3–1.9 μ (average 1.5 μ). H is a member of the Chlorophyceae, with green pigment, 5.5–7 μ long (average 6.4 μ); width 5–8 μ (average 6.1 μ); with four flagella of length 5.6–8.5 μ .*

In the large cultures of female *Calanus* more than half the animals generally remained healthy for 3 to 9 weeks, while a few were kept active for over 100 days, after which these cultures were abandoned (Table I). Nicholls (1933) states from an analysis of field data that the length of adult life of a female is about 6 weeks.

Only in two series of experiments (cultures C and G) was the survival poor. In the case of C, this might be attributed to the slightly high temperature employed (12° C.), though subsequent lowering of the temperature to 8° C. did not retard the mortality rate materially. The mortality in culture G may definitely be attributed to adverse conditions

* Flagellates B.II, D, C, F, and H were supplied to the Marine Station at Port Erin, and there used as food for the rearing of oyster larvæ. However, their description of Flagellate D as a Cryptomonad, 4.7 μ long, red (Bruce, Knight, and Parke, 1940), does not conform to our "D" as described above. Either the original culture of "D" has become contaminated and finally suppressed by the red flagellate in Port Erin, or the label "D" has been applied to a different species isolated locally. It is an unfortunate fact that most of these minute flagellates—if not all—have not yet been described, and that we cannot identify them; but as long as we have no better names, the preliminary labels attached to the flagellates by those who first isolated and cultured them should be respected in order to avoid confusion.

TABLE I.—THE SURVIVAL OF FEMALE *CALANUS* ON VARIOUS FOODS.

Expt. and Date.	Food.	Temperature, °C.	Average Survival: > 50% Healthy.	Longest Period of Survival for an Individual.
X, 10/6/38	<i>Chlamydomonas</i>	8.5° till 18/6; then 6.0°	18 days	> 45 days *
Yc, 22/11/39	<i>Nitzschia</i>	5-7°	58-64 "	82 " *
Ya, 22/11/39 } Yb, 22/11/39 }	<i>Chlamydomonas</i>	5-7°	(No counts made)	> 99 "
B, 15/2/40	<i>Skeletonema</i>	10° till 20/3; then 8.0°	60 days	70 "
C. I } C. II } 25/4/40	"	12° till 1/5; then 8.0°	6-7 "	22 "
D. III, 6/6/40	<i>Chatoceros</i>	5.0°	> 16 "	(Culture abandoned)
E. 2, 12/7/40	<i>Nitzschia</i>	6° till 1/8; then 10.0°	31 "	52 days
E. 1, 12/7/40	<i>Skelet. and Nitz.</i>	6.0°	19 "	{(Cultures abandoned after 50% dead)
E. 10, "	"	"	19 "	"
E. 12, "	"	"	24 "	"
E. 11, "	"	"	9-15 "	"
E. 3, "	<i>Chlamydomonas</i>	"	19 (> 75% alive)	"
E. 4, "	B. II	"	13-16 days	"
E. 5, "	<i>Chatoceros</i>	"	> 19 "	"
E. 13, "	<i>Skelet. and Nitz.</i>	6° then 10°	(No counts made)	> 55 days
E. 16, "	<i>Skelet., Nitz., and</i>	6° then 10°	"	> 72 "
E. 18, "	<i>Chat.</i>	"	"	"
H. I } H. II } 6/12/40	<i>Skeletonema</i>	9.5°	{All animals alive after 13 days and cultures then abandoned	

* Some Stage V copepodites included in this experiment.

previous to culturing, since after only 2 to 3 days large numbers of animals were dying off from a heavy parasitic infection. The survival period in Series E was also somewhat reduced, and this may have been related to the food cultures having at this time suffered from a bacterial infection, or to the fact that *Calanus* tends to die off during the late summer (*cf.* Fuller, 1937).

It was soon observed that although female and male *Calanus* were kept under precisely similar conditions (usually in the same culture dish), the males did not survive for so long a period as the females. The males would be healthy and active, and would successfully and repeatedly fertilise females in the culture, but the length of adult life appeared to be much reduced. Ruud (1929) suggested from field observations that male *Calanus* live for a shorter period than females. An analysis of the data from cultures containing only one male and one female (henceforth termed "pair" cultures) showed that, except in culture C (where survival of females has been already noted as very poor), of 24 cultures using the whole range of foods, females outlived the males in 17, and survived for equal periods in 4. The average period of survival for males was only 1 to 2 weeks (Table II). This result was fully confirmed

by data from the large mixed male and female cultures, and further in two series of experiments where separate large male cultures were set up to test survival specifically (Table III).

TABLE II.—DIFFERENTIAL SURVIVAL OF MALE AND FEMALE *CALANUS*.

"Pair" Cultures; Series E, July 1940; Series F, September 1940.

Expt.	Food.	Period of Survival (Days).	
		Male.	Female.
Ea.	<i>Skeletonema</i> and <i>Nitzschia</i>	12	Still alive *
Ec.	"	> 21	> 21
Ed.	"	20	19
Ea.I	<i>Ditylum</i>	2	Still alive *
Ee.	<i>Chlamydomonas</i>	8	"
Ee.I	<i>Ditylum</i>	3	"
Ef.	<i>Chlamydomonas</i>	> 17	> 17
Eg.	<i>Nitzschia</i>	12	Still alive *
Eg.I	"	3	"
Eh.	"	7	"
Ei.	<i>Chatoceros</i>	14	"
Ej.	B.II	17	"
Ek.	<i>Chatoceros</i>	14	"
El.	"	7	"
Fa.	<i>Skeletonema</i> and <i>Nitzschia</i>	Still alive *	7
Fb.	"	5	Still alive *
Fc.	<i>Nitzschia</i>	5	"
Fd.	<i>Ditylum</i>	6	"
Fe.	<i>Chatoceros</i> and <i>Nitzschia</i>	8	"
Ff.	<i>Chlamydomonas</i>	6	"
Fg.	E	Still alive *	1
Fh.	<i>Skeletonema</i> and <i>Nitzschia</i>	1	1
Fj.	<i>Nitzschia</i>	> 6	> 6
Fk.	"	2	Still alive *

Totals: Female outlived male—17; Male outlived female—3; Equal period of survival—4.

* Still alive when cultures abandoned.

TABLE III.—THE SURVIVAL OF MALE *CALANUS*.

Expt. and Date.	Food.	Number of Males.		Period of Expt. in Days.
		Initial.	Final.	
F.I, 7/9/40	<i>Skeletonema</i> and <i>Nitzschia</i>	15	5	3
E.6, 12/7/40	"	20	1	14
E.7, "	<i>Skelet.</i> and <i>Nitz.</i> till 21/7, then <i>Charoceros</i>	16	1	15
E.8, "	<i>Nitzschia</i>	20	1	14
E.9, "	<i>Skeletonema</i> and <i>Nitzschia</i>	24	7	13

The large male cultures were characterised by the very small production of faecal pellets, even when many males remained healthy and active for several days. Also in the "pair" cultures, in the few cases

in which the male outlived the female, the number of faecal pellets produced dropped very far below 50 per cent. of the original, immediately following the death of the female. The obvious suggestion that adult male *Calanus* were feeding much less actively than the females was tested in the following experiments.

II. The Feeding of Male and Female *Calanus*.

Healthy male and female *Calanus* were transferred, after being washed in sterile sea-water, to separate dishes containing the same quantities of medium and food, and were left to feed under identical conditions for a fixed period. Counts were made of the faecal pellets produced by this feeding, provided that the animals were still alive.

It will be seen (Table IV) that in 10 of the 11 experiments the females fed more actively than the males, although different diatoms and flagellates were used as food. The number of faecal pellets produced by a female was from 2 to 10 times as great as that counted in the male cultures. This, however, does not give a true estimate of the difference in the feeding rate, since in almost every experiment (except with Flagellate B.II) the faeces produced by the females were distinctly larger on an average than those formed by the males. Fig. 1 shows the usual type of faeces obtained in experiments on the feeding of males and females. Measurements were made on these faecal pellets, and considering them as approximately cylindrical, the average volumes were readily calculated. For *Skeletonema* and *Ditylum* the ratios female volume/male volume were 5.5 to 1 and 6.5 to 1 respectively. In experiment F.3, however,

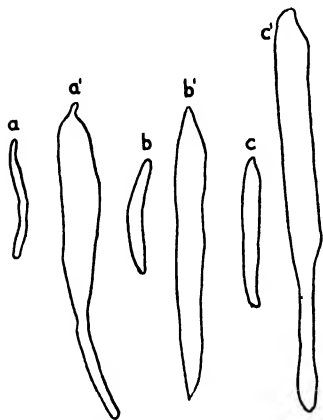


FIG. 1.—Faecal pellets of *Calanus finmarchicus*.

a—male pellet; a'—female pellet;

Food—*Skeletonema* and *Nitzschia*.

b—male pellet; b'—female pellet;

Food—*Skeletonema*.

c—male pellet; c'—female pellet;

Food—*Ditylum*.

(Camera lucida drawings, all $\times 30$.)

The majority of pellets obtained from females consist of a large main portion, and a narrower "tail piece" (a' and c'). This type of pellet was obtained with all kinds of diatom food, including *Skeletonema*. In the experiment from which b' was drawn, however, only the simple type of faecal pellet was produced. Pellets from males were always of the simple type.

using *Nitzschia* and *Skeletonema* as food, the faeces produced by the males were excessively small and few in number, and the female/male ratio in this experiment was as high as 50 to 1. From the data on *Ditylum* it has been calculated that a female consumed 19 *Ditylum*

cells in producing one faecal pellet, while a male takes 54 cells to produce the same volume of faeces. The figure for the female is almost certainly too low, owing to errors introduced in sampling, and, while no definite conclusion can be drawn from a single experiment, possibly about 50 *Ditylum* cells are consumed for every faecal pellet produced.

TABLE IV.—COMPARATIVE FEEDING OF MALE AND FEMALE *CALANUS*.

(Numbers in brackets refer to the number of faecal pellets per individual per 24 hours.)

Date.	Temp. °C.	Food.	Duration of Expt. in Hours.	No. of Animals.	Number of Faeces.	
					Male.	Female.
13/9/40	10	<i>Nitzschia</i>	47	1	119 (61)	91 (46)
19/9/40	11	<i>Skelet. and Nitz.</i>	21½	1	5 (6)	82 (92)
20/9/40	11	<i>Nitzschia</i>	21	1	0 (0)	89 (102)
18/9/40	11	<i>Chatoceros and Nitz.</i>	24	1	7 (7)	60 (60)
16/7/40	6-7	<i>Skelet. and Nitz.</i>	24	23	20 (1)	*800 (35)
18/7/40	6-7	<i>Skelet. and Nitz.</i>	24	23	120 (5)	1200 (52)
15/2/41	9·5	<i>Skeletonema</i>	24	1	41 (41)	119 (119)
15/2/41	9·5	<i>Ditylum</i>	24	1	18 (18)	34 (34)
15/2/41	9·5	<i>Nitzschia</i>	24	1	3 (3)	25 (25)
15/2/41	9·5	B.II	24	1	27 (27)	40 (40)
15/2/41	9·5	D	24	1	0 (0)	37 (37)

* Approximate count only.

The faecal count comparisons were confirmed by two experiments in which the initial and final food concentrations were also estimated in each culture. Thus 25 male and 25 female *Calanus* were allowed to feed on equal concentrations of *Ditylum* for 68 hours (initial concentration—506 cells per c.c.; temperature 6° C.). At the end of this period 24 females were healthy and only 20 males, but after allowance had been made for this differential mortality, there was still a very great difference in feeding rate between the two sexes: males produced 540 pellets and consumed only 31 cells per c.c., while the females produced 4000 pellets and consumed 476 cells per c.c. Considering the difference in size of the faecal pellets for *Ditylum* food also, the true female/male feeding ratio is in this case almost 40 to 1, but if only the number of pellets be compared, the ratio is as low as 6. The animals were closely examined at the close of the experiment, and, although the female culture had been swept almost clean of diatoms, many females still had green food in their guts, and a few showed green faecal pellets not yet voided. By contrast, in the male culture, although masses of food were available, all the animals except two had the gut empty.

In the second experiment, 15 males and females were allowed to feed

under precisely similar conditions on *Chaetoceros pseudocrinitus* (initial concentration—6300 cells per c.c.; temperature 6–7° C.) for five days, without changing the cultures. As was expected, the survival of the males was comparatively poor (4 males to 13 females alive), and the feeding rate for the males was low. Assuming a constant mortality rate for both sexes (and this actually *increases* the apparent feeding rate for the males!), and calculating on equal numbers (14) of both sexes feeding for five days, the following data are obtained:—

	Food Consumed, Cells/c.c.		Fæces Produced.	
	Males.	Females.	Males.	Females.
Results from experimental counts	1400	5450	670	2720
Calculated values for 14 copepods feeding for five days	2178	5529	1042	2759

The female/male feeding ratios obtained respectively from the calculated food consumption and faecal pellet production agree very well, viz. 2.5 to 1, and 2.6 to 1, but again the male faecal pellets were much smaller, and hence the true feeding ratio was approximately 15 to 1.

III. *The Feeding and Moulting of Stage V (copepodite) Calanus.*

During late summer and autumn the tow-nettings obtained showed a great preponderance of Stage V copepodites. (Presumably, the overwintering stock was being established—*cf.* Russell, 1928 and 1935; Marshall, Nicholls and Orr, 1933–35; and Farran, 1927.) A few experiments in 1939 had shown that Stage V copepodites would feed actively on *Nitzschia*, on *Chlamydomonas*, and also on the very small flagellate “D” for over three weeks, after which the experiment had to be stopped; a few moults had been obtained. It appeared desirable, following the work of Clarke and Gellie, 1935; Fuller and Clarke, 1936; Fuller, 1937; and Clarke and Bonnet, 1939, to test the survival of Stage V copepodites on varying known concentrations of *Nitzschia*.

The animals were taken from a stock, kept for four weeks and fed on *Skeletonema* and *Nitzschia*. Only very healthy individuals, thus accustomed to culture conditions, were selected. After being washed in sterile sea-water, batches of fourteen to fifteen animals were transferred to culture dishes with 200 c.c. of medium. In this experiment, Erdschreiber medium was soon replaced by sterile sea-water, as colloidal substances in the Erdschreiber were precipitated, and taken as food by the copepods to some extent. Some soil extract was, however, introduced with the food organisms, which have always been cultured in Erdschreiber. The

following concentrations of *Nitzschia* were used: F. 1—0 cells per c.c.; F. 2—1000 cells per c.c.; F. 3—10,000 cells per c.c.; F. 4—50,000 cells per c.c.; F. 5—200,000 cells per c.c.; F. 6—500,000 cells per c.c. They were renewed every 48 hours, when faecal counts were made, and any dead animals removed after examination for moult.

The moulting process proved to be a critical stage (*cf.* Fuller and Clarke, 1936; Fuller, 1937; Clarke and Bonnet, 1939) and no successful moults were obtained in this experiment. Cultures F. 1–F. 4 gave a total of 47 animals dead, of which only 16 died in moult. The number of faecal pellets per copepod produced in 48 hours never exceeded 8! Thus it would appear that the majority of *Calanus* were starving, although a few obtained sufficient food to attain the moulting stage. Cultures F. 5 and F. 6 gave a total of 28 deaths, of which 21 were in moult (*i.e.* 75 per cent. of the animals attained the moulting stage as against only 33 per cent. in Cultures 1–4). During the first few days of the experiment, with these higher food concentrations each copepod was producing up to 30 pellets per 48 hours, but after a week the faecal pellet production fell very greatly, and probably all the copepodites had then ceased feeding prior to the moult. Lucas (1936), however, in experiments on the feeding rates of mysids and copepods lasting for only two days, obtained a great reduction in feeding rate on the second day, apart from any moulting.

In an attempt to obtain successful moulting, a second series of experiments (F. I–F. VII) was conducted in September 1940, the temperature being raised to 10° C., and unmeasured but large quantities of food regularly being added, so that a considerable excess was invariably present.

A vast accumulation of faecal pellets was observed throughout the experiments, and the very active feeding as compared with the previous series of experiments was presumably correlated with the high percentage of animals reaching the moulting stage (72 per cent. attained the moult). Although the actual moult was again a critical stage, a few individuals moulted successfully (Table V). Only in F. II and F. VI did many deaths occur apart from the moulting process, and this might be attributed to the use in both these cases of a *Skeletonema* food culture, which was afterwards found to have a bacterial infection. That *Skeletonema* is a perfectly satisfactory food for the growth of Stage V copepodites was proved by the results from three cultures (Ha, Hc, and Hx) set up later (4/12/40) with a healthy *Skeletonema* culture. The experiment lasted for 14 days and gave the following result: of a total of 31 animals, 12 died in moult, and of the 17 healthy and active survivors, many were moulting. No individual was recorded as dying apart from moulting.

TABLE V.—THE GROWTH OF STAGE V COPEPODITE *CALANUS*.

Temperature 10° C. Date 7/9/40 to 2/10/40. 200 c.c. medium for each dish.

Culture.	Food.	Dead in Moult.	Dead not in Moult.	Dead,* "Doubt- ful."	Alive in Moult.	Alive as Stage V.	Success- ful Moult.	Total.
F. II	<i>Skelet.</i> and <i>Nitz.</i>	11	7	4	..	1	2 females	25
F. III	<i>Nitz.</i>	19	1	2	2	..	1 female	25
F. IV	<i>Chat.</i> and <i>Nitz.</i>	22	1	2	1	27 †
F. V	<i>Chlamy.</i>	12	1	4	1	8	..	27 †
F. VI	<i>Skelet.</i> and <i>Nitz.</i>	10	4	6	..	5	1 female	26
F. VII	<i>Nitz.</i>	14	..	1	..	9	1 „	25

* "Doubtful" indicates condition uncertain as regards moulting, owing to some decomposition.

† 1 individual lost.

IV. *The Spawning and Development of Calanus.*

Calanus spawned successfully under the experimental conditions and the eggs obtained from both large and "pair" cultures were transferred to separate small covered glass dishes of cooled Erdschreiber. The chief difficulties encountered in egg production were the relatively short life of the males, and, in the "pair" cultures, the time-lag necessary for the ripening of the gonads and the attainment of the right time in both individuals for the transference of a spermatophore. Eggs were obtained from adults fed on various foods—*Skeletonema*, *Nitzschia*, *Chaetoceros*, *Ditylum*, *Chlamydomonas*, and Flagellate B.II, and spawnings were obtained throughout the whole year, with the exception of October and November. Cultures with mature adults were unfortunately not available during these months, but there seems little doubt that eggs can be produced throughout the year under laboratory conditions.

The best egg-production was from February to April, eggs being constantly spawned by one culture (B) of 27 females and 3 males from 17th February until 4th April. This agrees fairly well with Nicholl's (1933) estimate of six weeks as the length of life for adult *Calanus*. In this culture, after the initial dying off of a few females (it has been already suggested that 20–25 individuals is the maximum population for 250 c.c. medium), the remaining individuals survived very well and produced large, but in general diminishing, numbers of ova for the first three weeks. The egg-production then fell to a very low level for two weeks, but afterwards recovered sharply before a final decline (fig. 2), and all the females had died before the end of April. The great majority of ova were fertilised

until towards the end of the spawning, by which time all three males had died. It should be noted that lowering the temperature from 10° to $6-8^{\circ}$ C. did not improve, but rather depressed the spawning, until 27th March, after which the temperature was raised to $10-12^{\circ}$ C.

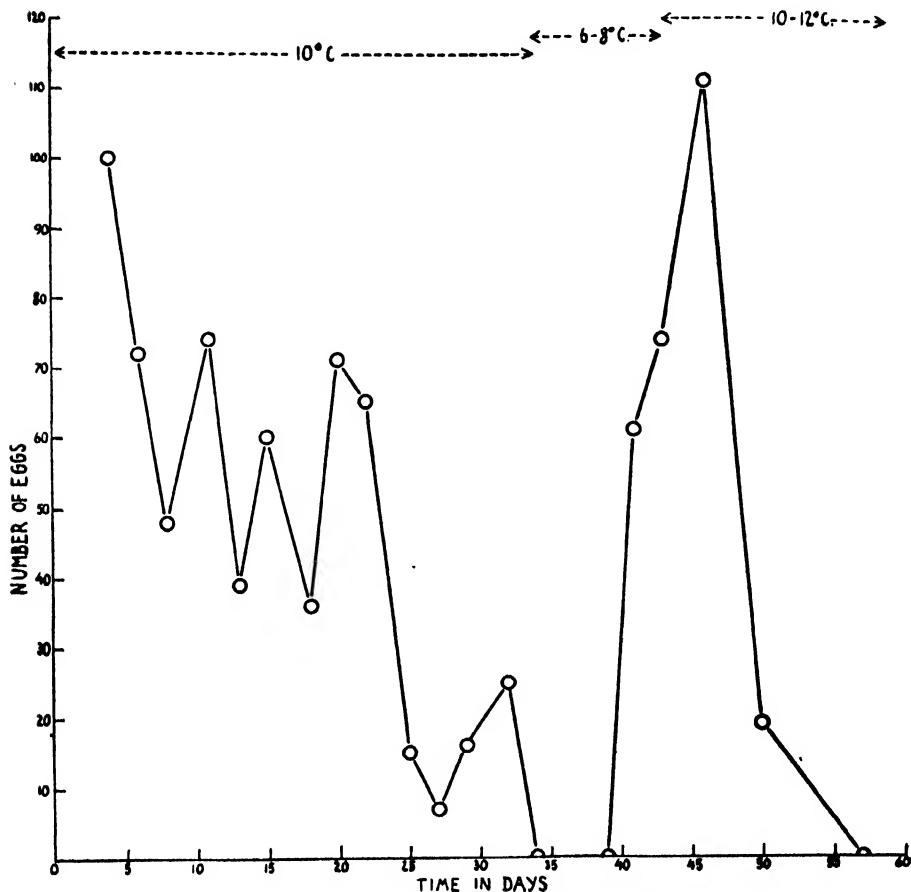


FIG. 2.—Egg production in *Calanus finmarchicus*.
Culture B. 2nd February–12th April 1940. Food—*Skeletonema*.

Of other cultures, Ya and Yb at $5-8^{\circ}$ C. produced comparatively few eggs. Culture C (12° C.) was notable for the rather poor survival of the adults (*vide supra*), and as might have been expected, the egg production in these experiments was also diminished. In an attempt to improve the production, the temperature was very gradually lowered to 8° C., but spawning decreased still further, and following a further drop to 6° C., not a single egg was produced. Similarly, Cultures E1–E5

and Ea-El (6° – 7°) yielded very few eggs, although a wide variety of foods was employed. Series D was also run at 5° C., and although reasonable numbers of ova were obtained during the first week, after that time production became almost negligible.

The method of oviposition is rather peculiar (*cf.* Marshall, Nicholls and Orr, 1934): one female was observed under the binocular microscope when spawning, and the ova, which were extremely small in the narrow oviducts, were seen to swell almost instantaneously after extrusion to form oval or slightly irregularly shaped eggs of very much greater volume; in fact each egg attained a volume greater than that of all the 30 to 40 eggs inside the oviduct, obviously by a rapid imbibition of water. Within about five minutes all the ova had become perfectly spherical and normal in appearance, except for a few opaque dead ones, which were probably not fertilised. Small eggs in process of enlargement (presumably just spawned) were also occasionally observed in other cultures. Both fertilised and unfertilised ova were always found on the bottom of the culture dishes, and they always sank to the bottom following transference. Mr Crawshay has confirmed this statement from his observations on the eggs of *Calanus* at Plymouth. Their specific gravity was obviously greater than that of sea-water (Gross and Raymont—following paper).

Data obtained from "pair" cultures showed that a female may spawn more than once. This confirms a suggestion made by Nicholls (1933) on the basis of unpublished results on *Eucheta norvegica*. Although many females spawned only once, Table VI shows that up to six spawnings were obtained within 16 days. A more careful analysis reveals that in many cases eggs were collected on successive days, and thus that in all probability a single spawning period was interrupted. When only undoubtedly distinct and separate spawning periods were considered, a maximum of three (possibly four) spawnings was found in from 13 to 21 days; the interval between successive broods may therefore be taken as from 4 to 7 days.

Nicholls (1933) states that there is no record of the number of ova produced by one female, and that it has not been possible to obtain this by experiment. Definite numbers obtained in spawnings may now be given from our results with "pair" cultures. The number of ova in any one deposition varied very widely (1–78), but while the maximum number ever obtained was 78, usually from 15 to 50 ova were deposited. It must be remembered, however, that a female can oviposit more than once. Our data on *total* egg-production per female are scanty, but Table VI shows that at any rate over 100 ova can be produced under culture conditions. Cultures Ce, Da, Df, and Di, in which egg counts

TABLE VI.—EGG PRODUCTION BY SINGLE FEMALE *CALANUS*.
(April–September 1940.)

Culture.	Dates of Separate Spawnings.	Number of Ova in one Spawning.	Total Ova Production.	Duration of Culture in Days.
Cb	7/5	10–15	10–15	5
Cc	26/4; 27/4	?; 40 (nauplii)	> 40 *	2
Cd	29/4; 7/5	?; 78	> 78 *	13
Ce	25/4; 27/4; 1/5; 2/5; 6/5; 7/5	?; 15–20 (nauplii); 33; 64; ?; ?	> 112 *	16
Cj	26/4; 7/5; 16/5	?; 16; ?	> 16 *	21
Da	8/6; 13/6	16; 41	57	13
Db	11/6; 17/6; 19/6	45; 17; 44	106	13
Dc	13/6	50	50	13
Df	9/6; 13/6; 17/6	?; 43; 15	> 58 *	13
Di	11/6; 13/6	18; 34	52	13
Eb	14/7; 16/7	3; 7	10	14
Ec	16/7; 17/7; 24/7; 29/7; 31/7; 1/8	3; 14; ?; 4; 6; 25	> 52 *	21
Ed	15/7; 17/7; 20/7; 24/7	16; 13; 28; 14	71	19
Ee	17/7	7	7	8
Ef	15/7; 17/7; 20/7; 26/7	18; 9; 10; 20	57	17
Ei	13/7; 16/7	4; 22	26	14
Fc	9/9; 11/9; 12/9; 13/9	10; 6; 15; 19	50	7
Fd	9/9; 11/9; 13/9	4; 7; 3	14	7
Fe	9/9; 11/9; 12/9; 13/9	5; 17; 20; 18	60	7

? = No count made though eggs were recorded.

N.B.—In all cultures on some days 1–2 eggs only were found. It is believed that this represented either the beginning or the ending of a spawning; thus they have not been regarded as separate ovipositions.

* The total egg-production is minimal, since counts were not made in some spawnings.

were most frequently made, suggest that large spawnings (40–50 ova) may perhaps alternate with smaller ovipositions (10–20 ova).

These data on spawning were obtained from cultures running from April to June. The spawnings in August and September (Cultures E and F) appeared to differ in that they extended over several days, and that only a few ova were deposited at any one time. Thus the maximum number ever deposited in these two culture series was only 28, and of 30 spawnings 25 gave less than 20 ova. But the *total* production by any female was still large—at least 70 eggs.

The eggs obtained developed satisfactorily and hatched in 24–48 hours. From a preliminary experiment it appeared that at a temperature of 8–6° C. nauplii hatched successfully and were subsequently very active, but that if the temperature was decreased to 5–6° C. the percentage hatch was considerably lowered, and the resulting nauplii tended to be inactive. Later experiments gave very successful hatching of active

nauplii at 10° C. On an average such larvæ survived for about a week (Table VII), many of them growing and moulting up to third or fourth stage nauplii (developmental stages—Lebour, 1916). This growth occurred with a variety of foods: pure cultures of the flagellates H, D, and F, and of the diatom *Nitzschia*, and also mixed cultures of Flagellate B.II and the diatom *Skeletonema*, etc. Nauplii were not, however, reared beyond the fourth stage.

TABLE VII.—SURVIVAL AND GROWTH OF *CALANUS* NAUPLII ON DIFFERENT TYPES OF PHYTOPLANKTON FOOD.

Culture.	Month, 1940.	Temperature, ° C.	Food.	Maximum Survival in Days.	Growth Observed.
B.1	Feb.	10	<i>Chlamydomonas</i>	< 7	—
B.2a	"	"	Ha	< 7	—
B.2b	"	"	<i>Nitzschia</i>	7	Growth to Stage III
B.2c	"	"	"Mixed" diatoms and flagellates	< 7	—
B.2d	"	"	"	7	Growth to Stage IV
B.3	"	"	D and F	5	"Some" growth
B.4	"	"	F	7	Growth to Stage III
B.5	"	"	<i>Skeletonema</i> and <i>Nitzschia</i>	< 7	Growth to Stages III and IV
B.6	"	"	Ha	4	Growth to Stage III
B.7	Mar.	"	D	> 7	Growth to Stage III
B.8	"	"	<i>Nitzschia</i>	< 7	Growth to Stage III
B.13	"	10 then 8.0	B.II and <i>Skeletonema</i>	> 10	Growth to Stages III and IV
Cb	Apr.—May	12 then 8.0	"	> 3	—
Cc	Apr.	12	"	4	Growth to Stage III
Cd	Apr.—May	12 then 8.0	D	> 3	—

DISCUSSION.

Previous experiments on the feeding of *Calanus* have been somewhat limited as regards the food organisms employed; only cultures of the very small diatom *Nitzschia closterium* var. *minutissima*, and of the flagellates *Platymonas*, *Chlamydomonas*, *Dunaliella*, etc., have so far been used, but neither *Nitzschia* nor these flagellates are normally represented in the rich phytoplankton flowerings which occur in the sea.

Now, the important theories of Hardy (1935) on "animal exclusion" and of Harvey (Harvey, Cooper, Lebour and Russell, 1935) on the "grazing" activities of zooplankton are based largely on observations on the relative abundance of zooplankton and of the typical planktonic diatoms, and it is therefore important to settle the question definitely as to how successfully copepods can utilise the common diatoms such

as *Skeletonema*, *Chaetoceros*, etc. Our results have shown that these diatoms are all most readily taken by *Calanus*, just as *Nitzschia* is taken. The adults survive and develop their sex products satisfactorily, while Stage V copepodites feed and grow successfully on these pure diatom diets. We have also found that the relatively very large diatom, *Ditylum*, forms a perfectly satisfactory direct food supply. Harvey (1937) also found that *Calanus* could filter this diatom successfully.

The well-known theory of Pütter on the utilisation of dissolved nutrient matter by the zooplankton has been much disputed, and in relation to the food requirements of copepods it has been emphasised that Pütter considered only the diatoms, neglecting the rich nanoplankton as a food source of the oceans. Clarke and Gellis (1935) and Clarke (1939), in discussing the importance of the nanoplankton as a *potential* food supply, have, however, admitted that scanty evidence is at present available as to how readily copepods can utilise the nanoplankton organisms. Marshall (1924), from an examination of the gut contents of *Calanus*, showed that the copepod fed on various kinds of diatoms, but the taking in of delicate naked flagellates cannot be demonstrated by such studies.

Our experiments with pure cultures of different autotrophic flagellates, ranging in size from larger forms like *Chlamydomonas* to very minute species (*e.g.* F and D), and all occurring in the sea, have demonstrated that such nanoplankton is also successfully utilised by *Calanus*—by the adults and Stage V copepodites, and also by the young nauplii. Attention has been drawn by one of us (Gross, 1937) to the existence of a very large and widespread group of minute autotrophic flagellates occurring in the sea, and to the fact that from single drops of sea-water cultures of several types may be started. However, our knowledge of this true micro-flora is still almost negligible in respect of classification, seasonal and regional distribution, reproduction, etc. The fact that copepods can feed on these minute species also, testifies to the extraordinarily efficient filtering mechanism possessed by these animals.

Thus, if we apply these results generally, it would appear that planktonic copepods can utilise as food a whole range of planktonic autotrophic organisms varying in size from at least $100\ \mu \times 50\ \mu$ (*Ditylum*) to $1.7\ \mu \times 1.2\ \mu$ (Flagellate D), and it is therefore probable that the successive flowerings of the different diatoms and of the various autotrophic flagellates do normally furnish an ample supply of food for the nutritional needs of the herbivorous zooplankton.

It is obvious from our results with cultures of male and female *Calanus* that most male individuals took little or even no food. While the mouth

parts in the males of *Calanus* are normal and some feeding does occur, in *Euchata norvegica* the oral appendages are much reduced (Sars, 1903), and thus the adult males presumably cannot feed at all. However, most *Calanus* survived for many days. It is known from the work of Fuller and Clarke (1936) that *Calanus* can live under starvation conditions, without growing, for about two weeks, and if it is supposed that the animal is then living on its fat reserves, the survival period for a starving Stage V copepodite can be estimated as from 9 to 19 days. Male *Calanus* in our experiments on an average survived for 7–14 days, and the survival period would thus appear to be largely determined by the food reserves.

But the males in our cultures were not only alive but were also actively producing spermatozoa, and thus one might expect some feeding to occur during this limited period of survival. The amount of reserve substances carried over from Stage V to Stage VI is probably the same for both males and females. In the female these reserves are largely used in egg production, but the manufacture of spermatozoa presumably makes much smaller demands on the food reserves, which, in the males, are therefore largely available for metabolism. Consequently, it is to be expected that male *Calanus* will have only reduced nutritional requirements during their short adult life.

Regarding the nutritional needs of Stage V copepodites, if we accept Fuller and Clarke's contention that the attainment of moulting by *Calanus* copepodites may be taken as an index of growth, it is obvious from our first series of experiments (F1–F6) that growth of Stage V copepodites was definitely poorer on the lower diatom concentrations. Clarke and Bonnet (1939) found that the best nutritional conditions were obtained with more than 30,000 *Nitzschia* cells per c.c., and this agrees with the results of our preliminary experiments. No experiments have been conducted with as high concentrations as 1,500,000 cells per c.c., with which Clarke and Bonnet found a harmful effect on *Calanus*. Even, however, with the higher diatom concentrations which we used, feeding in general appeared to be slow. It is possible that the comparatively low temperature (6° C.) at which these experiments were conducted depressed the filtering rate. Fuller (1937) found a much diminished feeding rate with *Calanus* maintained at 3° C. compared with others kept at 8° C. This idea was confirmed by the results of our later experiments, in which feeding was much more active at 10° C. and the great majority of the copepodites were seen to grow well.

While feeding and growth were satisfactory, a great mortality occurred during the actual moulting process. At first it was considered that the salinity of the Erdschreiber employed might have been too low (32.75

per mille), but experiments using medium of salinity 35.0 per mille failed to give better results. With our apparatus, small temperature fluctuations were unavoidable, and small variations in pH over the 24-hour period also undoubtedly occurred. We cannot entirely accept the statement of Clarke and Bonnet (1939) that the *Nitzschia* in their copepod cultures failed to alter the pH appreciably, since a series of observations *throughout* the 24-hour period might have revealed changes, sufficiently large to affect moulting animals. Atkins (1922) obtained a sharp rise in pH on exposing the alga, *Ulva latissima*, to sunlight in culture. A series of tests made recently on a very thick culture of *Nitzschia* showed an increase of pH from 8.78 in the morning to 9.74 in the evening, when kept near the north window, and from 8.90 to 9.33 when kept in the refrigerator and thus receiving very little light. It may be that during moults, though not at other times, these temperature and pH fluctuations become critical factors.

The results of our experiments do not support the suggestions of Clarke and Bonnet (1939) that Stage V copepodites do not feed so actively nor moult so readily towards the end of the summer, and that these differences are dependent upon seasonal phase differences in the life cycle. Our experiments, conducted in September and October, showed very active feeding and attainment of the moult by 72 per cent. of the animals cultured. The lack of success encountered in the previous experiments (F1-F4), conducted at the same season, we believe was determined by starvation, accentuated by the general depressing effect of the lowered temperature.

Although the moult is a critical stage, in our experiments moulting was definitely better at 10° C. Clarke and Bonnet (1939), however, claimed that a temperature of 6° C. was optimal for the process. It is, of course, quite possible that such lower temperatures may be optimal for *Calanus*, adapted to the presumably more rigorous conditions prevailing off the north-east coast of North America, but for individuals from the Clyde Sea-Area, a temperature much below 10° C. would appear to be detrimental to growth.

Calanus spawned freely in our cultures, but temperatures lower than 8-10° C. appeared to be less favourable for spawning. It is possible from the results of series D (at 5° C.), in which ova were successfully deposited, but only during the first week of the experiment, that low temperatures prevent the continuous ripening of the germ cells rather than depressing the actual process of oviposition.

Nicholls (1933) calculated from an analysis of field data that the number of ova spawned by any one female showed a very wide variation

(1-120), but he supposed that normally a female probably yielded from 60-70 eggs. In our experiments from 1-78 eggs were deposited, but we have shown that a female may spawn three or four times, and our figure of about 100 ova as the total egg production for one *Calanus* agrees well with Nicholls' calculated value. We have shown that in two culture series (E and F), set up in August and September, only very few eggs were spawned at any one oviposition, although the *total* egg production was still large. While series E was maintained at 6-7° C., F was set up at 10° C., and it would therefore appear that temperature was not producing this difference in spawning behaviour. The earlier experiments were carried out with spring-early-summer adults, while the animals used in series E and F belonged to a late-summer brood. Russell (1928, 1934, and 1935) has supposed that these broods differ physiologically, and it may be that they differ in their spawning habits. The small, somewhat irregular late-summer spawnings of *Calanus*, revealed by the field studies of Russell (1928), Farran (1927), and especially by the work of Marshall, Nicholls and Orr (1933-35), may therefore be a characteristic of the *individual* late-summer females, as well as being characteristic of the population as a whole. But, whether these differences in size, behaviour (and perhaps spawning) between the various broods are inherent constitutional characters, or whether they are determined by different environmental factors acting on the same hereditary material, is a problem that can only be solved when the continuous rearing of *Calanus* through several generations is possible.

The nauplii of *Calanus* fed and grew on a variety of small flagellates, and also upon *Nitzschia*, in our experiments. Since food was thus not limiting their survival, it appeared possible that the sea-water used in making the Erdschreiber medium (obtained from the Firth of Forth) was unsuitable; perhaps the salinity was too low. A series of egg cultures set up with Erdschreiber made from sea-water from the Clyde Sea-Area, and other cultures set up with sterile sea-water from the same source, however, actually showed poorer growth and survival. It was obvious when examining larvæ, that they were very sensitive to change in temperature (*cf.* Crawshaw, 1913), and it is possible that slight temperature fluctuations occurring in our cultures prevented further successful rearing (*cf.* also the high mortality among moulting Stage V).

Under laboratory conditions, larvæ were hatched throughout most of the year—from February to June, and in August and December. But of cultures D, E, and F (in each of which rather poor egg production was obtained), only culture D showed any successful hatching, and even in that culture very few nauplii hatched and these were limited to the first

few days. These three cultures were maintained at comparatively low temperatures (5° C. and $6-7^{\circ}$ C.), and this apparently inhibited the normal development. Many of the eggs showed no development and soon disintegrated; others formed embryos which failed to hatch, while a few developed into fully formed nauplii, which, however, died in process of escaping from the egg membranes. It must be noted that after a few days the temperature of culture F (and later that of culture E) was raised to 10° C., but no improvement in egg production or in hatching occurred. Possibly the previous low temperature had so adversely affected the maturing sex cells that the few eggs that were laid were non-viable.

A few experiments (cultures C) at 12° C. gave rather poorer hatching and growth of nauplii than cultures at 10° C., and it is thus tentatively suggested that a temperature of $8-10^{\circ}$ C. is optimal for egg production and for the development of those *Calanus* that breed about springtime in the Clyde Sea-Area. This agrees with the optimum which we postulated from our results for the moulting of Stage V copepodites, and it is interesting to observe that this is also the temperature range existing during April-May at the surface of the sea in the Clyde Area (see fig. 5, Marshall, Nicholls and Orr, 1934). Late spring and summer broods must, however, be able to spawn and develop successfully at higher temperatures ($10-15^{\circ}$ C.).

SUMMARY.

Calanus have been maintained successfully in cultures of Erdschreiber medium, under laboratory conditions. The copepods remained healthy and active for from 3 to 9 weeks, provided that the temperature was kept fairly constant. The young nauplii and also moulting individuals of Stage V copepodites were found to be less tolerant of slight temperature fluctuations.

Adult and Stage V copepodite *Calanus* fed on a variety of planktonic diatoms and also upon very minute autotrophic flagellates. The nauplii fed upon these flagellates and on the small diatom, *Nitzschia*.

Male *Calanus* were much shorter-lived than females, and this appeared to be directly correlated with the relatively very small amount of food taken by males, even when plenty was available. From a comparison of the number and size of faecal pellets produced by male and female *Calanus*, it was calculated that the female/male feeding rate ratio is as high as 40 to 1.

Adult and Stage V copepodites lived and fed at temperatures ranging from 5° C. to 12° C.; but spawning was apparently less prolific; hatching

and subsequent growth of nauplii was poorer, and moulting of Stage V copepodites was less successful at the lower temperatures (5–6° C.).

Female *Calanus* were found to be able to spawn three or four times. The maximum number of ova produced at any one oviposition was 78, but a female could produce a total of about 100 eggs.

Normal, viable *Calanus* eggs were small and somewhat irregularly shaped when first deposited, but they very rapidly swelled and became perfectly spherical. They hatched in 24–48 hours, and were reared up to the IVth nauplius stage, in 7 days, upon pure flagellate and on pure *Nitzschia* cultures.

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XXI.—**The Specific Gravity of *Calanus finmarchicus*.** By F. Gross, Dr.phil., and J. E. G. Raymont, B.Sc., A.M., Department of Zoology, University of Edinburgh.* *Communicated by Professor J. RITCHIE, M.A., D.Sc. (With One Text-figure.)*

(MS. received December 19, 1941. Read March 2, 1942.)

IN the course of our studies of reproduction of *Calanus finmarchicus* under laboratory conditions we noticed that all the eggs produced in our cultures were lying on the bottom of the glass dishes, and when transferred into fresh culture medium ("Erdschreiber") or sea-water sank down relatively very rapidly. As the eggs were fertilised and healthy, and a large proportion of them developed into active nauplii (Raymont and Gross, 1942), it was obvious that the density of the living egg was considerably higher than that of sea-water. No reference to this fact was found in recent literature on *Calanus*; on the contrary, the impression gained from records of field studies and of counts of eggs and nauplii collected from different depths was that the eggs were spawned and completed their development near the surface. Thus Kraefft (1910) states that larger numbers of eggs were mostly counted in samples from 5 to 0 metre than from greater depths—though the numbers given in his tables do not fully bear out this statement—while the nauplii were invariably much more abundant near the surface than below 5 m. Nicholls (1933) also found ova and nauplii to be most abundant in the top 30 m. Only Giesbrecht (1892) mentions, in a short paragraph on the ontogenetic vertical migrations of pelagic copepods, that their eggs seem to have a higher specific gravity than sea-water. He suggests that while they sink down the eggs pass through their embryonic development and as soon as the nauplii hatch these turn round and swim towards the surface.

The rate of sinking of *Calanus* was studied by Apstein (1910) and Gardiner (1933), but, so far as we know, neither the specific gravity of the eggs nor that of the adults has been determined. In view of the characteristic and important position of *Calanus* in the zooplankton community it seemed of interest to obtain at least some preliminary data,

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particularly as specific gravity has been shown to be one of the factors determining the vertical distribution of another planktonic species, *Daphnia pulex* (Eyden, 1923).

MATERIAL AND METHODS.

The observations recorded here were made on female, male, and Stage V *Calanus* received from the Marine Biological Station in Millport in the spring of 1940 and during the winter months of 1940-41, and maintained in culture for varying periods of time, and on eggs and nauplii produced during those periods in the laboratory. Owing to the very restricted space in the refrigerator where the cultures were kept the number of animals that could be used for the density determinations was relatively small, and only some of the eggs produced were available for this purpose. An advantage, however, lay in the viability and healthiness of the material used.

The specific gravity of female, male, and Stage V *Calanus* was estimated by determining the rate of sinking of the specimens in rectangular glass cells, 10 cm. high and 1.3 cm. deep, filled with different solutions of gum arabic of known density. These were prepared by dissolving the gum in sea-water and filtering it through cotton wool so as to exclude air bubbles. Before being placed in the gum solutions the animals were anæsthetised in solutions of chloretone in sea-water. Neither this nor the gum had any detrimental effect; the animals recovered rapidly after most experiments and could be used for repeated timing of their rate of sinking.

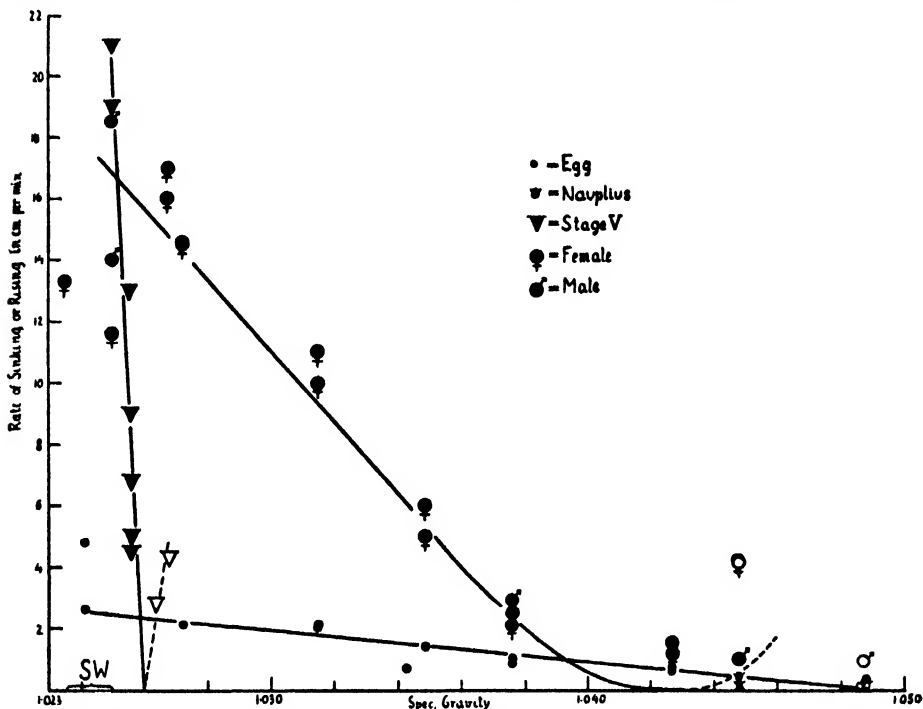
Because of the small numbers of eggs available the centrifuge method of determining their specific gravity (Lyon, 1907) could not be used, nor could the probably more accurate method recently worked out by Lowndes (1938) be used owing to the small size of our objects. Instead the eggs were transferred singly into either a glass cell or a watch-glass about 20 mm. deep, containing gum solutions, and their movement followed by means of a horizontal reading microscope or a low-power binocular respectively. The pipettes employed for the transference of eggs had their ends sharply bent so that the eggs were driven towards the surface of the solution before they began to sink. In solutions which proved to be of higher density the eggs were transferred with a straight pipette to the bottom of the dish and then allowed to rise.

The temperature was kept approximately constant at 13° C. for the eggs and nauplii by keeping the dishes containing the media in the refrigerator up to the moment when everything was ready for taking the

readings. The observations on the other stages were made at room temperature of about 15° C.

RESULTS.

As has been noted by Gardiner (1933) most *Calanus* individuals sink vertically, tail first, and with the antennæ fully extended. When the



Rates of sinking and rising of different stages of *Calanus finmarchicus* in sea-water (S.W.) and solutions of gum in sea-water of different density. Black symbols denote sinking, white symbols rising specimens.

antennæ were partially or completely folded the position of the sinking body varied considerably and some individuals sank head first; but no marked differences were observed in the times taken in sinking by the same specimen in successive readings when the antennæ were first folded and then, after renewed anæsthetising, extended.

The accompanying figure gives the rates of sinking and rising of *Calanus* eggs, of a few newly hatched nauplii, of Stage V copepodites, and females and males in sea-water and solutions of gum of different densities. Each point is the average of at least three readings taken with the same specimen. The values for the sinking velocity of eggs in

solutions of different density fall readily into a straight line, while those of the females and males show a distinct decrease in slope with increasing density of the medium. As to Stage V *Calanus* a slight increase of density from that of sea-water to the value of 1.0256 brought about a rapid decrease in the sinking velocity of the animals, while at a slightly higher concentration of gum (*i.e.* at a density of 1.02634) the animals remained afloat at the surface, and when pipetted down to the bottom of the dish rose towards the surface at a rate of 2.8 cm. per min. Thus the line representing the rate of sinking of Stage V shows a remarkably steep slope.

The point at which the line crosses the abscissa gives the approximate density of the respective stage of *Calanus*. There was, however, sometimes a considerable variation in the speed of sinking of the same specimen in successive experiments. Thus a Stage V *Calanus* sank through a distance of 9 cm. in a gum solution of 1.0256 density in 48, 32, and 47 sec. respectively. Different specimens showed sometimes even more marked differences in their rate of sinking. Thus two eggs sank in a gum solution of 1.047 density at a rate of 0.6 and 0.65 cm. per min. respectively, while a third egg drifted extremely slowly towards the bottom of the dish and then rose again to remain finally at the surface. After 15 min. the egg became obviously affected by the gum solution and it lost its transparency. The variation in the rate of sinking of different Stage V and female and male *Calanus* is shown in the figure.

For these reasons the following values for the specific gravity of the different stages of *Calanus* are only approximations:—

Eggs	specific gravity 1.045–1.049
Newly hatched nauplii	„ „ 1.045–1.049
Stage V <i>Calanus</i>	„ „ 1.0255–1.0265
Females	„ „ 1.043–1.045
Males	„ „ 1.043–1.047

There does not seem to be any difference in density between the eggs and nauplii or between females and males, and only a little difference, if any, between eggs and adults. There is, however, a significant difference between the Stage V *Calanus* whose specific gravity is only slightly higher than that of the sea-water used (1.0235–1.025) and all the other stages investigated. Unless the density of the animals increases in sea-water of a slightly higher salinity the density of Stage V *Calanus* will in many areas of the sea be as low or even lower than that of sea-water.

The velocity of sinking in sea-water is smallest in the eggs, *i.e.* about $2\frac{1}{2}$ cm. per min. The length of time required for the embryonic develop-

ment may be taken as 24 hours (Raymont and Gross, 1942). In the absence of currents the eggs will therefore sink down through a depth of 36 m. prior to the hatching of the nauplii. They are thus not endowed with the buoyancy of the eggs of many marine teleosts that under the same conditions would come up to the surface and remain afloat during the earlier part of their embryonic development. Their density equals that of the eggs of *Arbacia* and *Cumingia* which, according to Heilbrunn (1926), both have a specific gravity of 1.0485. (However, *Arbacia* eggs from animals collected towards the end of the season showed a higher density, *i.e.* 1.0656, while Lyon (1907) gives the specific gravity of unfertilised *Arbacia* eggs as 1.081–1.087.)

The sinking rate of the adults in our experiments, using sea-water of specific gravity 1.0235–1.025, was 11.5 to 18.5 cm. per minute, *i.e.* they would have taken 6 to 8½ min. to sink down through 1 m. The adult *Calanus* studied by Apstein (1910) showed a higher sinking velocity, *viz.* 1 min. 35 sec. to 5 min. 13 sec. (average 3 min. 21 sec.) per metre. Gardiner's Stage V *Calanus* took 4 to 8 min. to sink through 1 m.—which agrees with the time (approx. 5 min.) taken by our Stage V copepodites—while his adults showed a considerably higher velocity than our females and males and took only 2 to 5 min. to sink through 1 m. as compared with our 6 to 8½ min. Gardiner found that on the whole the time taken to sink through a fixed distance varies inversely as the length of the individual. As Stage V seem to be invariably smaller in length than adults (Marshall, 1933; Bogorov, 1934) and in our experiments sank more rapidly than the adults, Gardiner's conclusion does not hold for our material.

The density of 1.043–1.047 of the adults is much lower than that of *Labidocera aestiva* which Parker (1902) determined as 1.109 (a rather unexpectedly high value), but it is understandably higher than that of a fresh-water copepod *Diaptomus gracilis*, the density of which is given by Lowndes (1938) as 1.023.

DISCUSSION.

Our results demonstrated clearly that determinations of the sinking rate of animals in sea-water alone do not give more than an indication, if that, of their density. That the eggs sink much more slowly than Stage V, in spite of their higher density, is understandable in view of their much greater ratio of surface to volume (average diameter 190 μ). However, the behaviour of Stage V on the one hand and of the males and females on the other is rather surprising. There is no difference between their rates of sinking in sea-water such as we would expect from the

difference in their density. In all our experiments females and males sank more slowly in sea-water than Stage V, though the difference may not be very significant, in spite of their higher specific gravity. It seems, therefore, that these phenomena cannot be explained on the basis of Stokes's equation for the fall of a sphere.

The diameter of Stage V animals is presumably smaller than that of the adults and their specific gravity considerably lower. Hence their higher sinking velocity in sea-water can only be due to a smaller resistance offered to sinking by the shape and surface area of their body, perhaps the number and arrangement of bristles in particular, all of which contribute to what Ostwald (1902) called "Formwiderstand" and which he recognised as an important factor in determining the velocity of sinking of plankton organisms.

It is a striking fact that Stage V *Calanus* remains suspended in a medium of as low a density as about 1.0258, *i.e.* a density that is often found in samples of sea-water. Both Apstein and Lowndes used sea-water of a density of 1.027 for their experiments. Thus relatively very slight differences in the density of the sea-water may cause great differences in the rate of sinking of Stage V *Calanus*.

Russell (1931), discussing the vertical distribution of marine macroplankton, came to the conclusion that there are two factors inherent in the animals themselves which are largely responsible for the type of distribution shown during the night: (1) the depth at which the animal has been living during the previous daylight, and (2) the speed at which the animal is capable of swimming upwards. It seems to us that both factors are to some extent dependent on the specific gravity. The diurnal migration to greater depths caused by the increasing light intensity during daytime is effected either by swimming head downwards (*cf.* Russell, 1927) or by passive sinking, and this is clearly influenced by the specific gravity, though, as we have seen, not determined by it alone. The speed at which an animal is capable of swimming upwards depends primarily on the muscular power of its swimming appendages, but is influenced by shape and density. Suppose that Stage V and adult *Calanus* did not differ in shape but only in density; then their swimming strokes would carry Stage V farther than the adult, provided they expend the same amount of energy, or, in other words, Stage V will expend less energy to swim upwards through a certain distance than an adult.

This leads us to the consideration of another important point. It seems to be a well-established fact that in *Calanus finmarchicus* the continuation of the species is largely dependent on Stage V *Calanus*; it is they who tide the species over the winter months, when the number of

adults is at a minimum (Farran, 1927; Orr, 1934). So far as we know, no reason has been put forward to explain why *Calanus* passes the winter in the fifth copepodite stage rather than the adult stage. May the explanation not be found in the fact, indicated by our observations, that Stage V, owing to its remarkably low density, is the stage best adapted for the mode of life of plankton organisms? Orr (1934) showed that the fat content of both females and males is considerably lower than that of Stage V whose "high average fat content in the autumn and winter is puzzling when we consider that at that time plant food is scarce." The relatively very high fat content (22-45 per cent.) is, no doubt, largely responsible for the low specific gravity of Stage V *Calanus*, and obviously the low specific gravity, in its turn, enables them to make the most economical use of their fat reserves for the purpose of movements during the period of food scarcity in winter. Moreover, the increase of viscosity of the water caused by the lowering of temperature will tend to increase their buoyancy during that season even in sea-water of a lower density than their own and they will be able to maintain themselves in layers, offering the optimum conditions, at a minimum cost of energy. Once they have passed through their final moult their fat stores are presumably drawn upon heavily for the production of eggs and spermatozoa and are thus transformed into substances of higher specific gravity. No information is available about the density of the spermatozoa, but the fact that the eggs swell up and increase to many times their volume as soon as they have passed through the genital aperture (Marshall, Nicholls and Orr, 1934; Raymont and Gross, 1942) suggest that their density prior to spawning is very much higher than after the imbibition of water.

The wide seasonal variation in weight, size, and chemical composition of *Calanus* found in nature (Marshall, 1933; Orr, 1934; Marshall, Nicholls and Orr, 1934; Bogorov, 1934), and the differences between the rate of sinking of our material and that of Gardiner, suggest the possibility of considerable seasonal as well as individual variation in density of the different stages of *Calanus*. It may even be that in the sea their specific gravity varies at different periods of the day, as was found in *Daphnia pulex* by Eyden (1923). In order to establish such differences a much more comprehensive study would be required than could be attempted by us, but there is little doubt that such an investigation would yield results of considerable interest.

SUMMARY.

The specific gravity of eggs, newly hatched nauplii, Stage V, females and males of *Calanus finmarchicus* was estimated from the rate of sinking

of anæsthetised specimens in sea-water and solutions of different density of gum arabic in sea-water.

The eggs are not buoyant; they sink in sea-water at a rate of about 2.5 cm. per minute and their specific gravity is 1.045–1.049. The newly hatched nauplii and the males and females showed similar values, viz. 1.043–1.047, while the specific gravity of Stage V *Calanus* was found to be only slightly higher than that of the sea-water used, i.e. 1.0255–1.0265.

The bearing of the results on problems of vertical distribution is discussed. It is suggested that it is the low density of Stage V and the consequent economic use of its fat reserves that makes this stage suited best for tiding the species over the winter months.

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XXII.—Deficiency Effects of Ultra-violet Light in *Drosophila melanogaster*. By **B. M. Slizynski**, Ph.D., Institute of Animal Genetics, University of Edinburgh. *Communicated by* Dr A. W. GREENWOOD. (With Two Text-figures.)

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INTRODUCTION.

STADLER AND SPRAGUE in a series of papers (1936) succeeded in demonstrating by a genetical method the effect of ultra-violet radiation in maize pollen, and found that in their experiments in the X-ray series the chromosomal changes are very common, while in the ultra-violet series they are rare.

Recently Swanson (1940) carried out the first cytological analysis of the effects of ultra-violet light in *Tradescantia* and by direct comparison of the effects of X-rays and ultra-violet radiation on chromosomal changes confirmed Stadler's and Sprague's genetical findings.

Working on *Drosophila*, Altenburg as early as 1930 discovered the mutation-producing properties of ultra-violet light. A Notch, possibly a deficiency, was once obtained in 1931 (reported to Dr Muller in a personal communication) but "this may have been an accident unrelated to the treatment" (Muller, 1940).

Mackenzie and Muller (1940) have by genetical methods confirmed their former (1939) conclusion that ultra-violet light is ineffective in the production of gross chromosomal changes in comparison with X-rays. They suggest in a form of provisional conclusion, in view of the absence of Bar reversions or cases of Notch, that ultra-violet rays cannot produce minute chromosomal changes or at least they are far less effective in this respect than the X-rays, when equivalent in their mutation-producing strength.

In 1939 Dr H. J. Muller suggested the cytological analysis of the lethals obtained in the above experiments, and the present paper embodies the results of this analysis.

MATERIAL AND METHODS.

The exact details concerning the genetic constitution of the material and all physical data in regard to the dose applied have been given by

Mackenzie and Muller (1940), and therefore only a brief account will be given here.

Ultra-violet and X-ray lethals were obtained from Dr K. Mackenzie and Dr H. J. Muller, and the spontaneous lethals from Dr R. B. Singh. According to a personal communication from Dr Mackenzie, the dosage for ultra-violet lethals was: 3.6×10^{11} ergs/cm.² with a wave-length of 280–400 mμ, and for the X-ray series, 400 r units.

Out of 28 ultra-violet cases received, 21 were analysed cytologically, 7 being lost during breeding experiments. Out of 28 X-ray cases, only 7 reached the stage of cytological analysis (and of these one proved to be a translocation and was discarded), 17 were lost, and the remaining 4 cases were excluded beforehand from cytological analysis as having gross chromosomal changes. All the 8 spontaneous stocks were analysed cytologically, so that the total number of stocks analysed for deficiencies was 35.

All the stocks contained the lethal changes in the sex chromosome of the inverted *sc*⁸ type, and before cytological studies were undertaken the genetic localisation of the lethals was carried out, using a specially built up stock. The whole material in regard to its genetical constitution is divided into two classes, A and B; class A containing the gene for yellow body colour in the chromosome carrying the lethal change, and class B its wild type allelomorph.

The stocks of the genetic constitution: $\frac{y\ sc^8\ B\ w^a}{y\ Hw\ m\ g\ dl-49}$ and $\frac{sc^8\ B\ w^a}{y\ Hw\ m\ g\ dl-49}$ respectively were used for crossing-over experiments; females of the first type (class A) were mated to males carrying the characters *sn w*, and females of the second type (class B) were mated to males carrying *y sn w* genes.

The crossing-over in the *sc*⁸ chromosome has been studied extensively by Mather (1939) and the map distances obtained were as follows: *y*–5, *o*–*car*–10, *8*–*f*–22, *5*–*dy*–14, *9*–*ct*–3, *9*–*cv*–3, *o*–*ec*–1, *o*–*w*. In order to get comparable sections of the X-chromosome the following map distances interpolated from Mather's data were assumed: *y*–16, *1*–*B*–36, *1*–*sn*–8, *9*–*w*. These distances were used as a basis for calculating of crossing-over data and for localisation.

In order to obtain the highest possible degree of objectivity Dr Muller suggested a method of automatic checking of the cytological findings. The numbers of the stocks were changed without the authors' knowledge of their identity, and after localisation, when the slides were ready for examination, they were renumbered and divided in 60 batches without

TABLE I.—RESULTS OF CROSSING-OVER EXPERIMENTS.

Parental formulæ (lethal change in a Bar-chromosome)

Class A: $\frac{y\ sc^B}{sc^B} \frac{B}{sn\ w} \times y\ Hw\ m^B\ g^4\ dl-49$; Class B: $\frac{sc^B}{y\ sc^B} \frac{B}{sn\ w} \times y\ Hw\ m^B\ g^4\ dl-49$

No. of Stock.	Class.	Locus.	Cross-overs for Class A.																Total No. of Males.	Proportion of Females to One Male.
			y	..	y	..	y	..	y	..	y	..	y	..	y	..	y	..		
			B	..	B	..	B	..	B	..	B	..	B	..	B	..	B	..		
w ^a	w	w	w ^a	w	w ^a	w	w ^a	w	w ^a	w	w ^a	w	w ^a	w	w ^a	w	w ^a			
Cross-overs for Class B.																				
..	y	..	y	..	y	..	y	..	y	..	y	..	y	..	y	..	y			
B	..	B	..	B	..	B	..	B	..	B	..	B	..	B	..	B	..			
..	sn	sn	..	sn	..	sn	..	sn	..	sn	..	sn	..	sn	..	sn	..			
w ^a	w	w	w ^a	w	w ^a	w	w ^a	w	w ^a	w	w ^a	w	w ^a	w	w ^a	w	w ^a			

X-ray series:

107	B	"y"	..	62	..	3	..	37	..	10	2	..	10	124	3.35
117	A	10.4	3	85	10	6	..	60	1	9	1	3	178	3.37
122	A	19.6	2	82	19	..	5	48	..	17	2	..	2	..	1	5	183	2.46
104	B	36.7	3	53	7	..	10	6	..	5	3	..	1	..	2	90	3.17
123	A	60.3	..	134	24	..	68	..	22	1	..	3	2	4	..	4	262	2.27
108	B	"w"	..	25	5	..	17	..	10	3	1	61	2.47

Ultra-violet series:

83	B	0.8	..	77	2	25	..	54	..	16	..	8	..	5	..	6	193	3.14
78	A	15.9	..	109	9	39	..	11	7	..	2	5	182	2.44
71	B	17.2	..	86	14	..	2	47	..	19	12	..	8	4	2	..	194	3.23
76	B	18.9	2	74	10	..	3	36	..	8	9	..	10	..	1	2	155	3.06
75	B	20.3	..	64	8	..	2	28	..	9	1	..	2	..	2	1	177	2.40
87	A	22.4	..	57	15	..	6	29	..	4	4	1	116	2.51
63	B	24.8	2	61	14	..	11	32	..	23	6	..	4	..	2	2	1	..	158	2.03
68	B	26.1	..	57	5	5	..	11	..	1	79	4.00
80	A	30.5	2	48	11	..	17	31	..	8	1	1	2	..	4	1	126	3.70
84	A	30.8	2	61	11	..	20	24	..	18	5	1	1	142	2.20
86	A	31.0	..	136	22	..	22	37	..	15	..	1	3	236	2.53
88	A	31.6	5	72	13	..	22	28	..	10	4	1	2	..	1	158	3.34
62	A	32.2	1	101	23	..	26	26	..	13	9	2	2	..	1	1	205	3.08
69	A	33.2	1	61	21	..	25	25	..	14	3	1	1	..	1	1	1	..	155	3.76
61	A	35.6	5	95	23	1	35	34	..	23	4	1	5	..	11	1	1	..	239	2.40
77	B	36.7	2	71	18	..	33	26	1	23	4	1	1	..	6	186	3.10
81	A	41.7	7	95	22	3	37	17	..	15	1	2	..	2	9	2	..	1	213	2.00
65	B	54.9	..	42	12	..	21	..	3	9	..	1	3	2	2	1	96	3.75
82	A	59.6	..	118	25	..	40	..	14	1	..	3	1	1	1	204	2.07
79	B	"w"	..	89	16	..	52	..	8	4	..	3	..	10	182	3.14
74	B	"w"	..	62	11	..	25	..	10	3	..	2	..	5	119	3.02

Spontaneous series:

38	B	"y"	..	65	..	23	..	42	..	10	..	4	..	7	..	7	..	1	159	1.94
43	B	"y"	..	13	..	11	..	11	..	2	..	1	32	2.06
44	B	"y"	..	70	..	14	..	56	..	18	..	5	..	4	..	9	176	1.90
40	B	9.4	5	67	7	4	..	42	..	14	1	..	3	143	1.67
41	B	33.0	4	76	21	..	26	30	1	15	4	1	3	..	3	184	1.84
37	B	41.6	1	74	11	4	34	15	3	17	1	5	5	2	2	1	175	1.55
42	B	47.2	..	73	19	..	28	10	..	4	1	..	6	141	1.75
39	B	57.1	3	46	12	..	30	..	5	3	1	3	1	104	2.36

the authors' knowledge of the their original numbers. Therefore at the beginning of cytological studies the whole material consisted of 181 slides ordered in 60 batches belonging to 35 lethals; some of the lethals were represented by two batches, but their identity was not disclosed until the studies were finished.

The origin, genetic constitution (in classes A, and B), and genetical data for all cases are given in Table I.

Permanent salivary gland preparations were made of the female larvæ of the following genetic constitutions:—

$$\begin{array}{ll}
 (1) \frac{y \text{ } sc^8 \text{ } B \quad w^a}{sc^8 \quad sn \text{ } w} & (2) \frac{y \text{ } sc^8 \text{ } B \quad w^a}{y \text{ } Hw \text{ } m^3 \text{ } g^4 \text{ } dl-49} \\
 (3) \frac{sc^8 \text{ } B \quad w^a}{y \text{ } sc^8 \quad sn \text{ } w} & (4) \frac{sc^8 \text{ } B \quad w^a}{y \text{ } Hw \text{ } m^3 \text{ } g^4 \text{ } dl-49}
 \end{array}$$

In making the preparations, albuminised slides were used and the ordinary aceto-carmin and alcohol-euparal technique was employed. The observations were made with a 1.4 N.A. apochromatic objective 1/12" and 7x, 10x or 15x eyepieces with a substage condenser of N.A. 1.4 covered with immersion oil. An ordinary microscopic lamp with green filters was used. The drawings were made with the aid of camera lucida in 5000 diameters at the table level using the same optical outfit and a 30x eyepiece for general outlines; the details were drawn in later using a 15x or 10x eyepiece.

RESULTS.

The results of cytological analysis are summarised with all details in Table II, in which the cytological findings are grouped in three grades of certainty: (1) Suspected disorder; these sections are in brackets. (2) Probable structural changes, such as deficiencies ("df"), or inversions ("in"), where no conclusive pictures have been found, are in hyphens. (3) Certain deficiencies with exact definition and complete cytological evidence are in italics. The data in Table II under the heading "Cytological analysis" are represented as they were obtained, before the identity of batches was disclosed. The agreement or disagreement of the cytological findings among the batches belonging to the same stock shows the efficiency of the cytological analysis and the usefulness of Muller's self-checking method.

The results are also represented in the drawing (fig. 1). In the middle there is a genetical map of the X-chromosome based on interpolated Mather's data with marked "visible" genes as: y, B, sn, w below the line, and lethal stocks bearing their original numbers above it. Below

TABLE II.—SUMMARY OF THE CYTOLOGICAL ANALYSIS.

X-ray Series:					Ultra-violet Series:				
Original No.	Batch No.	No. of Slides.	Cytological Analysis.	No. of Bands Missing.	Original No.	Batch No.	No. of Slides.	Cytological Analysis.	No. of Bands Missing.
107	13 95	3 3	20 A 3 deficiency 20 A 3 deficiency	1 ,,	83	17	3	20 A 4, 5 deficiency	2
117	87 35	2 3	(11 E, 12 E, 13 A)		78	63 96	2 2	
122	16	3	(13 C)		68	74 90 94	2 2 2	-10 F 5 df- -14 D 2/1 in-	
104	54 32	3 2	(9 F) -9 F 8 df-		71	15 76	5 3	. -14 A 6, 7 df-	
125	81	3	3 C 8 deficiency	1	76	18	3	(13 B)	
108	66 52	3 2	/T (3 R : 2 L)/ /T (3 R : 2 L)/		75	26	3	
Spontaneous Series:					87	55 62	3 2	(inert 19 F, 17 A)	
					63	21 51	2 3	(12 E) -12 E 8, 9 df-	
					80	77	4	(11 D, 11 E, 12 E)	
					84	59 105	4 2	(11 C, 11 CD, 11 D)	
					86	69	4	11 D 9, 10 deficiency	2
					88	98 5	3 4	11 C 3 deficiency 11 C 3 deficiency	1 ,,
38	67 97	3 3		62	34	3	
43	70	3		61	28	4	(11 C 1, 11 D 1)	
44	48	3	20 A 3 deficiency	1	69	6	4	
40	2 25	2 2	(inert 16 F) (inert 16 F)		77	39 42	3 3	(9 F) 9 F 8, 9, 10 df-	
41	4 11 92	3 3 4	(inert 7 C, 9 EF)		81	24 33 100	2 3 3	(9 C, 10 A)	
37	82	3	(5 F)		65	65 102	2 3	5 F 3, 4 deficiency 5 F 3, 4 deficiency	2 ,,
42	14 56	3 2		82	58	5	4 C 3-16 deficiency	14
39	72 80	5 5	-4 E 2/1 in- -4 E 2/1 in-		79	83 91	2 3	(3 F 2/1 in) -3 E 1 df-	
					74	78	4	(10 E)	

In the stock No. 108 there was found a translocation involving autosomes 2 and 3, which give rise to two new chromosomes of the following structure (given in Bridges' sections): 2 sp-f-41-93-100, and 3 sp-f-81-92-40-21.

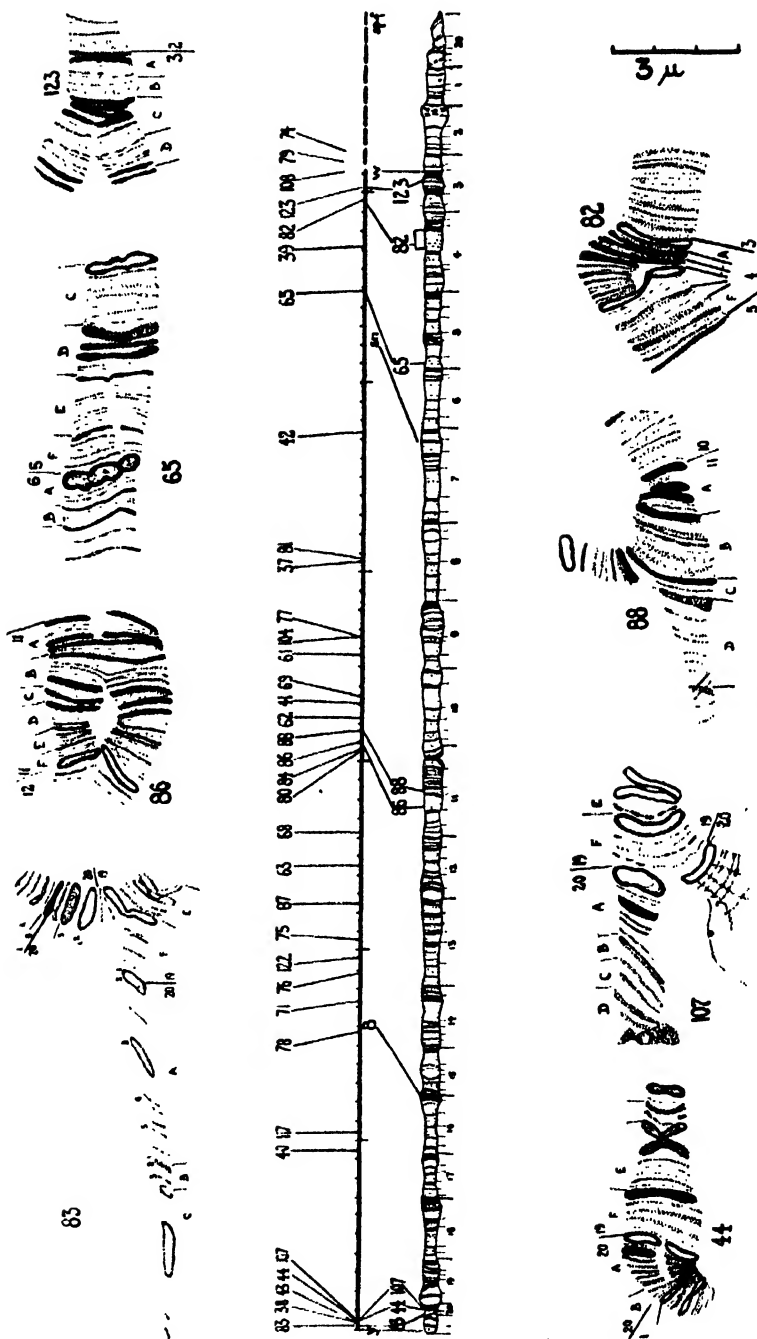


FIG. 1.—Summary of cytological analysis. (Full description in text.)

the genetical map there is a schematic drawing of Bridges' salivary gland chromosome map in inverted sc^8 position with the spindle fibre (sp-f) end to the right. The "visible" genes from the genetical map are connected with the corresponding regions in the salivary gland chromosome map. The lethals which have been found to be deficiencies are marked on the salivary gland chromosome map. On both sides of this diagram there are drawings for each of the 8 detected deficiencies numbered with their corresponding original numbers.

Lethal No. 107, belonging to the X-ray series, shows a deficiency of 20 A 3, a double slightly vesiculated band. The sc^8 chromosome beyond 20 A 4, 5 diffuses in the nucleolus. The nucleolus organising centre lies probably at 20 B 1, 2 or even beyond it. This agrees with Kaufmann's indications (1938) that the organising centre of the nucleolus is situated between 20 B 1, 2 and 20 C 1, 2.

In case No. 117 the two batches differ in the results of cytological analysis; in batch No. 87 no change had been found, while in the other batch there were some indications for disorder in sections 11 E, 12 E, 13 A, but none of them proved real. In the case No. 122 there was only one batch consisting of 3 slides and there were some suspicions for a disorder in section 13 C. In the case No. 104 for which there were 5 slides ordered into two batches, in the first batch the section 9 F was suspected; the suspicions are strengthened by the results of examination of another batch and were substantiated as suspected deficiency for the section 9 F band 8. In case No. 125 there was one batch with 3 slides in it; the deficiency was found with exact definition for one band 3 C 8. In the last case of X-ray series, No. 108 consisting of two batches, no morphological abnormality was found in the X-chromosome.

In case No. 83 of ultra-violet series the preparation from which the drawing was made, was from a larva of the constitution $sc^8/dl-49$. The delta -49 chromosome is very well extended and shows additional bands which were not reported by Bridges (1938), namely, very pale bands between 20 A 1, 2 and 3 and 20 A 6. The band 20 A 3 shows vesiculated structure and 20 A 4, 5 are both distinctly double. The deficiency removes 20 A 4, 5 and probably 20 A 6. In the sc^8 chromosome there are two vesicles corresponding to 20 A 1, 2 and 20 A 3, followed by 20 B 1, 2 and then by 1 B 1, 2. The deficiency removes at least 20 A 4, 5, *i.e.* two bands, if Bridges' map is taken as standard, or according to this picture, 5 bands, namely: 20 A 4a, 20 A 4b, 20 A 5a, 20 A 5b, and 20 A 6. In the following cases, Nos. 78, 75, 62, 69, no abnormality was suspected. The case 68 showed in one batch suspected deficiency for 16 F 5, in the other an inversion in 14 D 2/1, and in the third batch there were

no changes seen. Similarly in case No. 71, in one batch there were no changes seen, while in the other the section 14 A 6, 7 was suspected as an inversion. In case No. 76 there was a very slight suspicion of an undefined disorder in the section 13 B. In case No. 87 in one batch no changes were seen, while in the other some kind of "inertness" was suspected in the sections 19 F and 17 A. In case No. 63 the slight disorder in one batch in the section 12 E was more certain in the other batch as a deficiency for 12 E 8, 9. In case No. 80 there was a great variety of suspected alterations (inversions, deficiencies?) ranging from the section 11 D through 11 E to 12 E, but none was certain. In case No. 84 in one batch nothing was found, while in the other the section 11 C or 11 D was suspected. In case No. 86 a clear deficiency of two bands, 11 D 9, 10, was found. Similarly in case No. 88 in two batches a well-defined deficiency of one band 11 C 3 was observed. In case No. 61 there were some suspicions for 11 C 1 or 11 D 1. None of them proved real. In case No. 77 in one batch the section 9 F was suspected, while in the other batch the suspicion grew more certain for a deficiency of three bands, 9 F 8, 9, 10. In case No. 81 in one batch the section 9 C or 10 A was suspected, while the remaining two batches showed no change. In case No. 65 in two batches a deficiency for two bands, 5 F 3, 4, was found and exactly defined. In case No. 82 the largest deficiency was found, removing 14 bands in the section 4 C. In case No. 79 in one batch an inversion of the bands 3 E 2/1 was suspected, while in the other batch the deficiency was probable for the band 3 E 1. In the last case of the ultra-violet series, No. 74, there was a slight suspicion for an undefined disorder in the section 10 A.

In the spontaneous series in the cases Nos. 38, 42, and 43 nothing was found. Lethal No. 44 shows a deficiency for a half of the vesiculated band 20 A 3; it is impossible to determine which part of it, whether the proximal or distal, is missing. In case No. 40 in both batches the section 16 F contained "inert" material showing the tendency to associate with the chromocentre. This tendency has been observed in some other cases but not with such constancy, anyhow some sections sometimes showed marked affinity with the heterochromatin characterised by pairing, which sometimes was quite unspecific; these were the sections 12 E and in lesser degree 11 A. In case No. 41 there were slight suspicions of inertness in the section 7 C and 9 EF, but in the remaining two batches nothing like this has been observed. In case No. 37 the section 5 F was suspected. The last case, No. 39, showed in both batches strong evidence of an inversion of a vesiculated band 4 E 2/1. This case very likely will prove to be real.

All gross chromosomal changes observed during the present study were found only in the X-ray series. As already mentioned all such cases have been excluded from the cytological analysis.

Out of 11 cases of X-ray induced lethals only 6 were without gross structural changes in the X-chromosome. The remaining 5 cases were 3 translocations and 2 inversions. Exact data concerning the changes involving the X-chromosome are given in Table III.

TABLE III.—GROSS STRUCTURAL CHANGES, INVOLVING THE X-CHROMOSOME, DETECTED IN THE X-RAY SERIES.

No. of Stock.	Type of Change.	New Chromosome produced described in Bridges' Sections.
157	T (X : 2L)	1-12 C-30 D-41-sp-f2.
158	T (X : ?)	Lost.
164	In (X)	1-7 A-13 B-7 B 1-20-sp-f1.
170	In (X)	1-9 A 1-20 A-9 A 2-20-sp-f1.
149	T (X : 3 R)	1-17 C 4-81 F-100-17 D 1-20-sp-fX.

The sex ratio index (presented in the last column of the Table I) for spontaneous lethals shows almost normal deviation from 1 : 2 ratio. The figures of X-ray induced lethals as well as those for ultra-violet ones show that they are composed of two separated groups. The lower group reaches its maximum at the sex ratio 1 : 2.53, then there is a large gap up to the sex ratio 1 : 3. The other group starts from 3.02 and climbs up to 4.00, reaching that peak in the ultra-violet series. This seems to indicate that there is more than one lethal change induced in the case of high sex-ratio index. The more of such changes and the wider they are apart from each other the higher the sex index.

In summarising, it may be briefly stated that 6 X-ray induced lethals were analysed, among which there were two deficiencies. Out of 21 lethals in the ultra-violet series, 5 were detected as deficiencies, and, finally, out of 8 spontaneous cases there was 1 deficiency.

The results agree fairly well with my former results (1938) on the cytologically detectable deficiencies in X-ray and spontaneous lethals, when it was shown that 40.7 per cent. of X-ray induced lethals (or 16 per cent. if only those cases are counted which were checked by C. B. Bridges, M. Demerec, and B. P. Kaufmann, *Genetics*, 1938, p. 289) and 30 per cent. (or 12 per cent. with the above condition) of spontaneous lethals were associated with detectable deficiencies.

Qualitative structural changes are entirely absent in the ultra-violet as well as in the spontaneous series.

DISCUSSION.

The data represented above offer three main points for discussion. They are: the distribution of lethals and the size of the deficiencies. The third point is connected with differences between X-rays and ultra-violet light with regard to their effects on the structure of the chromosome.

The Distribution of Lethals.

The distribution of lethals may be regarded as a source of information concerning the nature of lethal changes. If, for instance, it were proved that lethals do occur preferentially in some regions or even at some particular points of the chromosome, this might lead to some conclusions about the stability and resistance of the structure of certain loci.

The detailed map of the distribution of lethals (fig. 2) suggests that they tend to accumulate at both ends of the chromosome and in its middle.

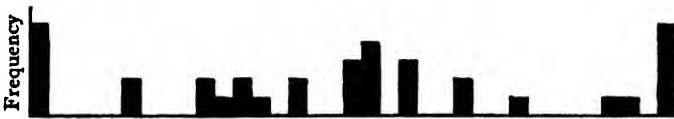


FIG. 2.—Diagram showing the frequency distribution of lethals.
Genetical map of *sc*⁸ chromosome.

The author is greatly indebted to Dr A. C. Aitken for supplying a method by which the probability of distribution of changes can be estimated, so that inference can be made as to the randomness or otherwise. In the material there are 35 changes occurring along a line of length 61.1 units. Supposing that we divide this range into 35 equal parts, each of 1.75 units, then the distribution of the 35 changes is as follows: 5, 0, 0, 0, 0, 2, 0, 0, 0, 2, 1, 2, 1, 0, 2, 0, 0, 3, 4, 0, 3, 0, 0, 2, 0, 0, 1, 0, 0, 0, 0, 1, 1, 0, 5. The most probable distribution is that in which there is just one change in each of the 35 subdivisions. It is most probable, but indeed it is of exceedingly small probability. Even so the above distribution has a probability which is about $\frac{1}{400,000,000}$ of that of equal distribution. It is thus almost certain that the hypothesis of randomness can be rejected.

The above calculations of Dr Aitken do not include the proximal part of the *sc*⁸ chromosome, which covers all the loci from the right of yellow up to the left of white; in the normal chromosome this section measures only 1.5 units. The sections taken into account end at the neighbourhood of *w*, leaving out the entire proximal part of euchromatin.

On the whole the distribution of lethals seems to be a non-random one.

There is no indication of differences in the distribution between the X-ray, spontaneous, and ultra-violet lethals; the numbers of the cases are too small. The same applies to the distribution of deficiencies for which no conclusion could be drawn, which might be used in discussing the relation between deficiencies and lethals so far as their distributions are concerned.

The Size of Deficiencies.

All deficiencies reported so far by different authors seem to have a limited but wide range of variation in size; they extend from 1 to a maximum of 50 bands, *i.e.* from $\frac{1}{1000}$ to $\frac{1}{20}$ of the total number of bands in the whole X-chromosome. These are the true deficiencies where the absolute number of bands has been diminished, all other cases connected with translocations, or insertions of the removed part into another chromosome, have not been considered. The upper limit of 50 bands for a deficiency in the X-chromosome is probably connected with the phenomenon of genetic balance of the nucleus.

The size of the deficiencies detected in the present study varies from a single band up to 14 bands. Four of these deficiencies (3 C 8, 5 F 3, 11 C 3, and 11 D 9, 10) affect very pale bands; three occurred in 20 A which is partly heterochromatic, and the last deficiency (4 C 3-16) starts with a heavy band, but all the remainder consists of very pale bands. The "halving" of a band occurred in 20 A 3, which although drawn by Bridges (1938) with distinct indications of its double nature was considered by him to be single. Deficiencies described by the author in his previous paper (1938) involve 7 cases, among which there were three one-band deficiencies and four two-band ones. These were 4 A 4, 10 B 8, 14 B 6 and 4 A 1, 2, and 5 C 4, 5 and 5 D 3, 4, and 7 E 9, 10. Two of these deficiencies affect heavy bands (4 A 1, 2 and 5 D 3, 4), all others occurred in pale bands.

Very recently Demerec and Fano (1940) described 48 cases of X-ray induced deficiencies not connected with any structural changes and 13 cases of spontaneous deficiencies also free from such changes. They found that the range of size variations in the X-ray series was from 1-50 bands, while in spontaneous from 1-13 bands.

Any consideration of lethals must take account of a whole range of types of changes.

The first type is represented by some change in the structure of a gene

which affects its activity in such a way that normal development is disturbed thus causing death. These lethals are sometimes called allelo-morphic lethals, obviously being undetectable in salivary gland chromosomes. There are several cases of Notch "deficiency" acting genetically exactly as all other Notch deficiencies, but showing no cytological deficiency (Gottschewski (1937), Slizynska (1938), and Demerec (1939)).

The second type consists of lethals which are directly connected with a deficiency. A loss of a gene removes one band or part of it in salivary gland chromosomes. Probably only a small fraction of these lethals may be detected cytologically. Deficiencies of more than one band remove almost certainly more than one locus. They may be with regard to their size more or less easily detected in salivary gland chromosomes. Their lethal action is of a compound nature. In this connection it is interesting to recall Poulson's observations on the action of N-8 deficiency in embryonic development. Cytologically it removes 18 bands (Slizynska, 1938) and its lethal effect in male embryos is connected with profound changes. Segmentation is greatly reduced and contraction of the germ band does not occur. The embryo contains very little or no endoderm and mesoderm. Poulson concludes that while the missing part of the chromosome in the case of N-8 is not essential for the formation of cells and the production of the blastoderm, its presence is necessary if the germ layers are to be produced normally and take part in the subsequent development.

The third type includes visible losses in the salivary gland chromosomes which are not lethal to the organism. Such cases are not very numerous, but still they may serve as an example of deficiencies of genes which under normal laboratory conditions are not absolutely essential to the life of the individual—nothing is known about their importance to the life of the species in nature. Non-lethal deficiencies have been described by Muller (1935) and by Demerec and Hoover (1936).

The Mechanism of Origin of Deficiencies.

Generally speaking the deficiency can originate by one of the following processes:—

(a) Spontaneous or induced loss of the autocatalytic capacity or reproductive power of a gene. This process can be taken as explaining the origin of single gene deficiencies. The deficiencies, including a group of genes, are more difficult to explain in this way since they would require some kind of chain mutation.

(b) Unequal crossing-over may lead to a deficiency and corresponding

duplication of a very few units, and, although it is known to occur with definite frequency in some regions, its general application meets with several difficulties.

(c) Two simultaneous breaks close to each other may lead to a deficiency or inversion. Such deficiencies do not differ radically from gross structural changes, such as translocations, segmental interchanges, etc.

(d) Another possibility involves the spreading of the effects of a single "hit" along the chromosome. This may perhaps account for different sizes of deficiencies according to the strength of the hit and probably to its spreading correlated with differences in regional structure of the chromosome. Darlington and Upcott (1940) reject this explanation as far at least as spontaneous changes are concerned.

Extensive discussion on the origin of deficiencies is to be found in Muller's paper (1940). In paragraph 8, dealing with the effects of ultra-violet light, Muller discusses two possibilities: (1) If ultra-violet light could be found to produce detectable structural changes, then it would be evident that they are not connected with a thoroughgoing chromosome breakage but occur within the unbroken chromosome sheath. (2) If according to the second possibility ultra-violet light does not produce any detectable changes, the ultra-violet mutation might be considered as associated with some kind of process taking place within the gene.

The results of this study do show that ultra-violet light produces in *Drosophila* deficiencies which are cytologically detectable and that, moreover, in considerable proportion, but that as far as it is known it does not produce any gross structural changes.

Muller is prepared to accept the existence of the chromosome "sheath" which is not breakable by ultra-violet light. This presumption would meet with some difficulties because of the large size (up to 14 bands) of deficiencies produced by ultra-violet treatment. The deficiencies of more than one locus occurring inside of this hypothetical "sheath" would require some kind of spreading of the destructive wave through consecutive loci for a remarkable long section of the chromosome.

Dr Helen Slizynska suggested a working hypothesis (partly resembling in some respects Belling's hypothesis of crossing-over published in *Genetics*, 1933), based on the disturbances in the mechanism of production of a new gene string. According to this hypothesis ultra-violet light may retard the production of the new gene or prevent it altogether, but it does not sever the longitudinal intergenic connections or prevent their formation. This may be yet another explanation of the origin of one-gene deficiencies. For deficiencies of larger groups of genes, two such changes occurring near each other could be assumed. In this case

it is probable that the longitudinal intergenic connections may be established in such a way as to leave out the entire intermediate portion of the gene string. When, on the other hand, the two changes are not near enough the linear connections are re-established and we have simply two mutations, in many cases so near to each other that they are practically undetectable as two by genetical means. When they are still farther apart two single deficiencies or mutations may be produced.

In discussing the origin of deficiencies, the mechanical and structural conditions of the chromosomes at the time of their production must be kept in mind. The chromosomes are coiled and to some extent twisted, this twisting of the chromosome may bring some genes closer to each other. These and other mechanical conditions may facilitate the elimination of a group of genes if the action of ultra-violet light caused any discrepancy in the time of formation of the new elements. If this were the case, the production of a deficiency involving a comparatively large group of genes would not require the occurrence of two changes.

All changes in the chromosomes may be divided into two groups: gene mutations and structural changes. The latter are more likely to involve some changes in linear connections than the former since there are many structural changes not associated with any lethal or visible effect. Sometimes these two types of changes may coincide. Demerec (1937) found that when a lethal effect and a chromosomal structural change occur in the same chromosome, one breakage point is at the place of the lethal change in 92 per cent. of cases. These lethals are probably deficiencies. It is so in the X-ray material. In ultra-violet experiments so far no such structural changes have been found, and it is probable that their frequency will be similar to that of spontaneous structural changes.

Ultra-violet light produces visible gene mutations, lethal gene mutations, and deficiencies with a considerable frequency (if it produces any structural changes like translocations, etc. they will be probably rather rare), therefore the assumption seems to be justified that it does not act on linear intergenic connections.

This assumption may perhaps gain certain support from the results of Caspersson (1941), who by ultra-violet spectroscopy found that the salivary gland chromosomes consist among others of two chemically different elements: (a) nucleic acids or their nucleotides which show very high absorption and form the bands being associated with the chromomeres, and (b) the proteins with vastly lower absorption which form the substance between the bands. Although it is almost certain that the genes are not composed of thymonucleic acids, still the fact of

the difference in absorption suggests that the genes may be attacked by ultra-violet light.

By the loss of a gene or the delay in its formation the linear intergenic connections may become diverted, this being facilitated by the particular topographical situation during the division of the linear connection into two at the end of the resting stage when the nucleic acids begin to attach themselves to the genes.

Mackenzie and Muller (1940) treated young adult males not older than 24 hours. This indicates that the changes induced by ultra-violet light occurred in the chromosomes of the mature sperm. According to Slizynska's hypothesis the deficiency in an ultra-violet experiment arises when the new gene string is being formed. If this is the case no deficiency should arise in mature sperm, but it may, and indeed should, arise in the next division (the first cleavage division), if we assume that some genes were changed in such a manner that their reproduction has been delayed or stopped altogether; then after the first division there will be one cell with a deficiency and the other without it, but with a gene changed in such a way that in every following reduplication it will constantly produce a deficiency for its locus. In the second generation of cells there will be three cells with a deficiency and one without it, in the third generation the proportion will be 7 : 1, in the fourth 15 : 1, and so on.

McClintock's paper (1941) deals with mechanical breaks of the chromosomes originated in an anaphase or telophase spindle figure produced by interlocking or doubling of the ring chromosomes in maize. These breaks are, generally speaking, followed by the fusion of broken ends.

Similarly, the X-ray induced breaks are followed by the fusion of broken ends, thus leading to all sorts of structural changes.

The breaks induced in the chromosomes after the ultra-violet treatment seem to be different. Swanson's data (1940) and observations show that the breaks are not followed by fusion of broken ends of the chromosome. "It becomes apparent," writes McClintock in this connection (1941), "that the capacity of a broken end to fuse depends upon the method by which the chromosome becomes broken and the conditions of the cell following breakage."

Sax (1940) assumes the existence in the chromosome of natural breaking-points which when broken show the capacity of fusion. The chromosomes broken at non-natural breaking-point lose their capacity of fusion, resulting in terminal deficiency.

"The method by which the chromosome becomes broken" (McClintock, 1941) may be interpreted as based on the nature of the place of break.

The chromosomes consist of protein fibres to which at their specific points "the chromomeres are attached desoxyribose or thymonucleic acid" (Darlington, 1942). The breaks occurring in the protein fibre between the specific points are not connected with gene mutations, but may lead to structural changes such as inversions, translocations, etc., which therefore have no visible or lethal effects. On the other hand, the changes occurring in the specific points of the protein fibre may lead to mutation, intercalary or terminal deficiency, the latter under certain conditions of the cell. The fusion in case of intercalary deficiency may occur beyond the destroyed specific points.

Sax (1940) reported that a very small fraction of breaks induced by the X-ray treatment appear to have lost their capacity of fusion. It is not known whether this healing was a permanent one, since the chromosomes were not observed in the following divisions. This very small proportion of breaks might represent these cases in which the X-rays destroyed the specific points.

The change occurring after the ultra-violet treatment takes place in the newly forming gene string when its freedom of movements is naturally limited. It gains more freedom only when all linear connections are completed—in other words, when all chances of a change without a "thoroughgoing" chromosome break have disappeared. Moreover, the extent of the change thus produced will be kept within certain limits controlled by mechanical and structural conditions existing at the time of the origin of the deficiency.

These size limitations as far as their order of magnitude is concerned agree fairly well with Demerec's and Fano's (1941) determinations of the radius of action of the "single event." These authors found that large deficiencies are less frequent in spontaneous than in the X-ray induced Notches. Their calculations show that 15 bands, corresponding to 6μ in the salivary gland chromosome in Notch region represent approximately the upper limit for a "single event" deficiency, while the deficiencies beyond this size very likely originate by the same mechanism as gross structural changes. They calculated the radius of action of this single event to be about 600 Å. The deficiencies detected during the present study do not exceed the limit of 15 bands, although the largest (14 bands) corresponds to 7μ on Bridges' (1938) standard map of salivary gland chromosome.

The differences between X-ray induced structural changes on one side and ultra-violet induced as well as spontaneous on the other may perhaps be explained with the aid of the above hypothesis. The X-rays being a very powerful factor cause changes of all kinds in all structural

elements of the chromosome and probably at all times; they may act on genes inducing visible mutations, lethal gene mutations, deficiencies which are cytologically detectable, they may destroy the continuity of the gene string producing all kinds of structural changes like translocations, inversions, insertions into heterochromatin, they may attack the centromere causing changes in its mechanism and in its division, they may even delay or stop the division of the whole nucleus, and so on. The ultra-violet light, on the other hand, seems to have a much narrower range of effects. According to the above hypothesis its main point of action is the gene itself, it produces visible gene mutations, lethal gene mutations, it can interfere with normal duplication of the gene thus causing its deficiency, but apparently it does not act on the intergenic connections.

Therefore if any gross structural changes should be found in an ultra-violet experiment, they are expected, on the basis of this hypothesis, to be always connected with a deficiency at the point of breakage.

SUMMARY.

35 cases of lethals in the *sc*⁸ chromosome of *Drosophila melanogaster* were analysed genetically and cytologically. Out of 6 cases of X-ray induced lethals, 2 were found to be connected with deficiencies, in 21 cases of ultra-violet lethals 5 were shown to be deficiencies, and among 8 spontaneous lethals there was one deficiency. Out of total of 11 cases of X-ray produced lethals, 5 were connected with gross chromosomal changes, while no such changes were found in 21 cases of ultra-violet lethals. In the spontaneous series there is one probable case of inversion.

The distribution of lethals seems to be a non-random one. They tend to be grouped in three groups—distal, medial, and proximal.

It is suggested that some of the X-ray, as well as of ultra-violet lethals, may contain more than one lethal change, the spontaneous cases seem to be single changes.

A hypothesis is put forward in order to explain the origin of deficiencies, which are produced by X-rays and ultra-violet light or originate spontaneously. According to it the ultra-violet light acts on genes only producing visible gene mutations, lethal gene mutations, and mutations which delay the formation of a new gene thus rendering it deficient.

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XXIII.—Types of Development of Polytene Chromosomes. By
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Merton, London. *Communicated by* Dr P. C. KOLLER. (With
Three Text-figures and One Plate.)

(MS. received May 2, 1942. Read July 6, 1942.)

1. INTRODUCTION.

UNTIL a few years ago the small size of the chromosomes limited the methods which could be used for their physical and chemical study. The discovery in 1933 that the salivary gland nuclei of Diptera contained giant multiple or polytene chromosomes threw the field open to the application of a variety of new techniques.

Dipteran larvæ vary widely, but are alike in that after a very early stage they grow chiefly by increase in cell size rather than by the more usual method of cell multiplication. This increase in cell size is accompanied or perhaps conditioned by nuclear growth. The chromosomes in the nuclei of particular tissues of larvæ before pupation are many times the size of those in other insects, and more clearly distinguishable. It was discovered that the chromosomes, while retaining their linear organisation, increase in size by repeated longitudinal division to give the banded polytene structure (Koller, 1935; Cooper, 1938). They may grow up to 300 μ long and 15 μ wide. In their chemical as well as their morphological differences they correspond with the small mitotic chromosomes (*cf.* Darlington, 1942).

My investigations were begun in order to make use of polytene chromosomes for the study of molecular structure by X-ray photography. They were carried out in conjunction with Dr W. T. Astbury, Textile Physics Laboratory, Leeds, and Dr C. W. Metz and Dr J. B. Buck, Carnegie Institution of Washington, Department of Embryology, Baltimore, U.S.A. The present paper contains data collected during these investigations, and concerns the structural peculiarities of polytene nuclei in Chironomidæ and related families.

2. METHODS.

(i) *Culture of Chironomus*.—The aquatic larvæ of *Chironomus* (Bloodworms) are found everywhere, and if no particular species is needed

there is no difficulty in obtaining supplies from outdoor throughout the year, except during the first warm spell in spring when the winter larvæ have hatched out and no summer larvæ have developed.

The adults were reared in aquaria measuring 15" × 12" × 12", containing a thin layer of mud (sterilised if necessary) and two inches of water, and covered by a muslin cage of the same dimensions. Though flies could be developed on nothing but unsterilised water and soy-bean meal, successive healthy generations were never obtained in captivity on this diet without the addition of mud. Unsatisfactory culture conditions lead to an increase in the time of development and to the failure of the larvæ to develop hæmoglobin. A succession of developing larvæ can be maintained by keeping them at 8°–10° C. until needed, and then transferring them to 16°–26° C. The lower temperature is preferable, as the difference in rate of development is not large and the cultures are cleaner and healthier at 16° than at 26°.

(ii) *Dissection*.—It is usual to dissect small larvæ of insects in Ringer solution, but *Chironomus* contains enough body fluid to make an artificial solution unnecessary. The body fluid in which the glands are normally bathed is more satisfactory than any artificial solution; but as it becomes increasingly alkaline on exposure to air (Boche and Buck, 1939) the glands were never left in it longer than a minute. Before dissection the larvæ were dried enough to prevent dilution of the body fluid by external water. When the temperature was above 20° C. an ice-cooled stage was used for dissecting the glands of the larvæ. Above 20° C. the dissected glands rapidly deteriorated and uniform results could not be obtained.

(iii) *Fixatives*.—For the study of polytene nuclei and chromosomes, smear preparations were made, stained with acetocarmine. The slides were made permanent by Bridges' vapour method (Bridges, 1937). The Feulgen staining is lengthier, and the chromosomes are less clear because of the high absorption of the dye, which obscures the finer details of chromosome structure. Smears stained with 1 per cent. orcein in 45 per cent. acetic acid give excellent detail with no background staining (Darlington and La Cour, 1942).

3. SPECIES EXAMINED.

CHIRONOMIDÆ: *Tanypodina*.

Pentaneura monilis, L.

Anatopynia trifascinnus, Zelt.

Anatopynia varius, Fabr. and spp.

CHIRONOMIDÆ: *Tanypus* spp.

Procladius spp.

Orthocladina.

Cricotopus sylvestris, Fabr.

Diamesina.

Prodiamesia olivacea, Mg.

Chironomina.*

Chironomus dorsalis, Mg.

Chironomus dorsalis, var. *venustris*.

Chironomus cingulatus, Mg.

Chironomus riparius, Goet.

Chironomus tentans, Fabr.

Chironomus plumosus, L.

Chironomus cristatus.

Chironomus decorus.

Chironomus lobiferus.

Chironomus pedellus.

Chironomus spp.

Endochironomus sp.

Glyptotendipes glaucus, Mg.

TIPULIDÆ: *Tipula* spp.

MYCETOPHILIDÆ: *Sciara* spp.

SIMULIDÆ: *Simulium* spp.

4. TISSUES WITH POLYTENE CHROMOSOMES.

The degree of polyteny found in the tissues of a dipteran larva varies with the species, and is also correlated with the cell size, rate of development, and metabolic activity of the tissue. Polytene chromosomes were once thought to be characteristic of ephemeral gland cells only. Such chromosomes are also found in non-secreting tissue. Cooper (1938) and Makino (1938) give lists of secreting and non-secreting tissues in which they observed polytene nuclei. In addition I have seen them in the nerve cells of the larger *Chironomus* larvæ. The large nuclei in the anal gills of *Chironomus* also have banded chromosomes, but they are of a low polyteny.

* The Chironomid species examined were North American and British; American species collected were identified by Dr H. K. Townes of Cornell, the others by the late Dr F. W. Edwards of the Natural History Museum, London.

5. UNEQUAL POLYTENY.

In *Sciara*, equivalent chromosomes in all nuclei of the main part of the salivary gland are of about the same size. In *Chironomus tentans*, about half the nuclei are slightly larger than the other half. Occasionally a gland is found where a single nucleus has chromosomes twice the diameter of those in the other nuclei, which condition is brought about by the doubling of chromosome material in that nucleus once more than in the adjacent cells. A similar condition was observed in *Drosophila*.

In one larva of *Anatopynia varius* (Tanypodinae) each of the nuclei in the salivary glands had a giant "IV," the other chromosomes being normal (Pl., A). There is no similar case recorded in salivary glands, but it is analogous to the situation in the "sex-ratio" type of *Drosophila* (Darlington and Dobzhansky, 1942), in which the X chromosome at meiosis in the male divides twice, instead of once as do the autosomes.

6. VITELLOPHAGE NUCLEI.

The vitellophages, or yolk nuclei, in the eggs of insects have been described as exceptionally large and as dividing "amitotically." It has not been noted before that they have diplochromosomes similar to those found in the perilem of *Spinacia* and after suppressed mitosis or meiosis elsewhere (Gentscheff and Gustafsson, 1939; Barber, 1940). *Chironomus* eggs have vitellophage nuclei of very high polyploidy; the chromosomes are grouped in bundles, like those described by Berger (1938) in the mid-gut of *Culex* at metamorphosis. If *Chironomus* mid-gut is comparable to *Culex*, this supports the view that the mid-gut, or part of it, is derived from the vitellophage nuclei (Johannsen and Butt, 1941), and the Diptera should provide excellent material for a settlement of this much-disputed point. It would be interesting to discover whether the diplochromosomes are carried over from the yolk cells to the mid-gut without alteration, or whether they break up to form normal nuclei—which would explain the observations of "amitosis"—these nuclei again increasing in chromosome number. In any case, having diplochromosomes these nuclei cannot be supposed to arise from the polytene nuclei of the larval mid-gut.

7. FUSION OF HOMOLOGUES.

In fully-grown larvæ of *Chironomus*, the homologous chromosomes in a polytene nucleus are usually fused so closely that there appear to be only four chromosomes, not eight, which is the diploid chromosome

number. The individual chromosomes are distinguishable only in heterochromatic regions, or when one of the homologues contains an inversion or translocation. In two related families, Tanypodinæ and Orthocladinæ, the homologues are coiled round one another and can be distinguished along the greater part of their length. In regions where the homologues are more widely separated, each homologue sometimes

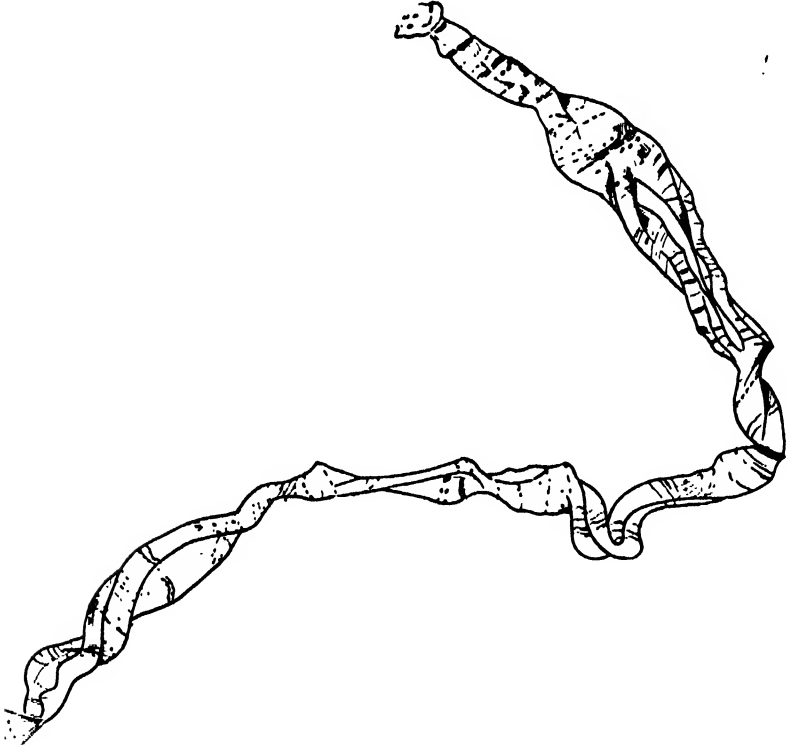


FIG. 1.—A polytene chromosome of *Anatopynia varius*; four threads showing in the region where the homologues are not closely associated. $\times 250$.

appeared to be made up of two strands coiling round each other (Text-fig. 1). Such a "four-strand stage" has been described by Painter and Griffen (1937) in mature *Simulium*. Buck (1937) in *Sciara* and Frolowa (1938) in *Drosophila* have also described a similar phenomenon found only in young larvæ. If, when the two strands divide, they are bound together, each pair would behave as one thread and the halves would not be detectable, but if separated, the strands in each pair would be free to twist. This twist therefore remains visible in the fully developed polytene stage of loosely associated chromosomes, although undetectable in species with close association.

8. HETEROCHROMATIN.

In ordinary mitotic nuclei in the Diptera as elsewhere heterochromatin is distinguished by its excessive nucleic acid charge during the resting stage, as shown by the Feulgen reaction. In the salivary glands of *Drosophila* this situation is reversed and the heterochromatin goes short of nucleic acid, a condition which is correlated with reduced cohesion of its daughter genes in bands, causing the loosening up of chromosome structure. Such a reversal in nucleic acid charge can be produced at metaphase of mitosis and meiosis in some plants and animals. Darlington (1942) has suggested that the heterochromatin is either super-charged or sub-charged, according to the variations of total nucleic acid supply within the cell, and of the demand for it by the euchromatin. Comparison of the salivary glands in *Chironomus* species suggests that a similar reversal occurs within the genus. In corresponding sub-median positions on all three long chromosomes, I find in different species either (1) a nucleic acid super-charged band (*C. plumosus*), or (2) a "puff" (*C. lobiferus*), which is a loose sub-charged region, described by Metz (1938) in *Sciara*, or (3) a nucleolus (*C. tentans*) (text-fig. 2).

This apparent relationship in position of heterochromatin and nucleoli in *Chironomus* agrees with what we see in the Diptera generally: where nucleolar organisers are present they lie in the heterochromatin. It should be noted that in the flowering plants there is no connection of position between nucleolar organisers and heterochromatin.

In the other groups examined heterochromatin is always sub-charged in respect of nucleic acid, but in different families it varies greatly in amount. *Sciara* is almost entirely euchromatic and it has no nucleolus. In most of the *Chironominae* the non-terminal heterochromatic regions are very small and fusion of the heterochromatin of non-homologous chromosomes occurs only at the ends. *Drosophila* has a nucleolus with an organiser lying in a heterochromatic region. A similar condition is found in some of the Tanypodinae and in *Prodiamesia olivacea*, the heterochromatic regions tending to be fused. In *Tipula* there are so many fused heterochromatic regions that it is impossible to distinguish the chromosomes as units: the nucleus appears as a network, the mesh of which is made up of short lengths of euchromatin, and resembles a magnified resting nucleus more than a polytene one.

In *Chironomus* with little heterochromatin, the nucleolus in some species is developed to an extraordinary degree (Pl., E). In extreme cases the longitudinal threads do not continue visible through the nucleolus. It is worth noting that the dissected chromosomes of *C. tentans* are firm,

and those of *C. plumosus* are loose, in structure, which may be correlated with the different amount of nucleic acid charge of the chromosomes in these two species respectively.

9. CORRELATED PROPERTIES OF POLYTENE CHROMOSOMES.

Chironomidæ.—The basic chromosome set consists of three pairs of long and one pair of short chromosomes. The short pair is always referred to as "IV." The different genera in section 3 and below are given in order of increasing polyteny in the salivary glands, which is accompanied by increase of uncoiling, pairing, and nucleic acid content.

Tanypodinæ.—The majority of species have two chromosomes attached to a large nucleolus, one sub-terminally and the other terminally, and two, including chromosome "IV," free. "IV" is very variable within the group and may only be a small lump. The chromosomes are relatively long, and the homologues do not fuse completely.

Orthocladinæ.—There is no visible "IV." There is no nucleolus, but heterochromatic regions occur on the three chromosomes, two of which are connected by heterochromatic fusion. Fusion of homologues does not occur until the fourth and last instar.

Diamesinæ.—"IV" is star-shaped, and the large nucleolus is at the end of one of the large chromosomes. Occasionally all three large chromosomes are attached to the nucleolus. Chromosomes are not coiled, though the homologues are fused, as in the *Chironominæ*; in gross morphology the polytene chromosomes of *Diamesinæ* are nearer to the *Tanypodinæ*.

Chironominæ.—The polytene nuclei consists of one long chromosome, two of medium length, and the short "IV." The chromosomes are always separate from each other and the nucleolus is always on "IV," which is longer than in other groups. In the different species of *Chironomus* there is a corresponding region, nearly median, on each of the longer chromosomes with the nucleolus, "puff," or extra strong nucleic acid band referred to in section 8. The homologues are fused at an early stage and are shorter, thicker, and less coiled than in the other groups.

When the chromosomes of different species within the *Chironomidæ* are compared, it can be seen that, with the increase in polyteny:

- (1) The amount of coiling left in the chromosome decreases.
- (2) Fusion between homologues is closer.
- (3) Nucleic acid charge increases.
- (4) The proportion of the chromosome which is heterochromatic decreases.

The correlation is not so close between different families of the Diptera, but chromosomes with a similar charge of nucleic acid always have a similar amount of fusion between homologues; and chromosomes of the same order in polyteny always have an equivalent degree of relic coiling. This applies even when tissue of one species is compared with different tissues of corresponding polyteny in another; for example, the gut cells of *Chironomus tentans* have chromosomes of similar size to those found in the salivary gland of *Drosophila* and the chromosomes are equally coiled.

10. MORPHOLOGY OF THE SALIVARY GLAND.

The order in which the Chironomidæ examined have been placed, on the basis of development and differentiation of polytene chromosomes, proves to be the same as that based upon the classification by the general morphology of adults. It is interesting to note that the comparative study of the morphology of salivary glands in the larvæ of Chironomidæ also leads to the conclusion that the Orthocladinæ and the Diamesinæ are intermediate between the Tanypodinæ and the Chironominæ.

The simplest possible gland might be taken to be a flat circular body with the cells forming an outer wall of uniform thickness. From this imaginary type an increase in secretion area could be obtained either by (a) increasing the volume of the gland with increase in the number of secreting cells, or (b) increasing the area of each cell in contact with the lumen. In (a) the circle can elongate to (1) an oblong, as found in *Sciara* (Doyle and Metz, 1935; Metz, 1935). This oblong is considerably extended in some other Mycetophilidæ; Madwar (1937) describes the presumably contorted salivary glands of *Brachypeza radiata* as being two and a half times the length of the body of the larva. The lumen can also be increased by turning the circle into (2) a sphere, and almost spherical glands are, in fact, found in the Tanypodinæ. The nuclei in *Tanypus* are distributed all over the surface of the globular gland (Pl., B). As the theoretical circle is elongated to form the *Sciara* gland, so in *Drosophila* we find the sphere elongated into a cylinder. The three-dimensional equivalent of *Brachypeza* is found in *Simulium*, where the glands extend the length of the larval body and are doubled back about a third of their length.

The Chironominæ have developed their secreting area by the second method (described above) (text-fig. 2). Not only do the cells of the salivary gland increase enormously in size (compare *Chironomus* and *Drosophila* nuclei, text-fig. 3), but the inner surface of the cell becomes convoluted; the lumen increases in size at the expense of the cytoplasm (text-fig. 3, A-D).

The cells almost fill the young glands (Pl., C), but as the larva develops the lumen encroaches until it surrounds each nucleus, sometimes completely. The cytoplasm is reduced to a thin outer layer of the gland and an even thinner layer around the nucleus, with, generally, a stalk of cytoplasm connecting the two (text-fig. 3, C). The nuclei in Malpighian tubules of *Chironomus* also protrude into the lumen.

Chironomus has far fewer cells in its gland than *Simulium*. The increase in the capacity for secretion was brought about by the enlarge-

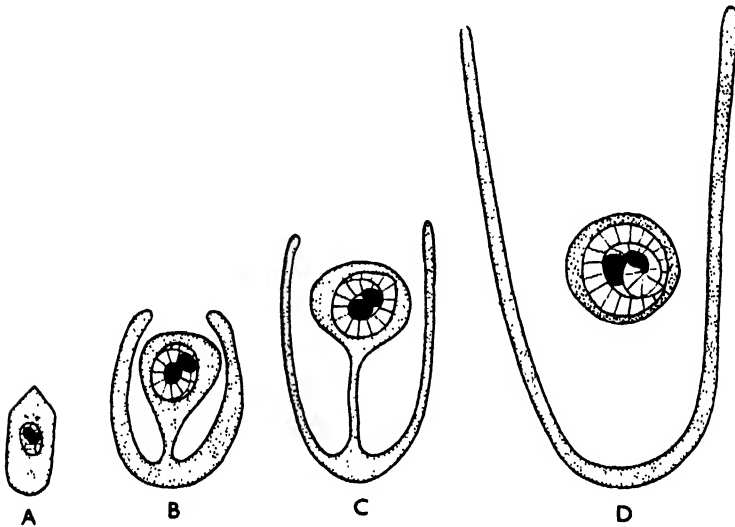


FIG. 2.—Diagrams showing the development of salivary gland cell in *Chironomus tentans*. In species with small larvæ the development stops at state B or C.

ment of cells. These are able to grow without distending the larva because the gland is flat, not spherical. Efficiency is further increased by increasing the area of the nucleus in contact with the lumen.

Prodiamesia olivacea has an unlike pair of glands. I have found that the glands of *Cricotopus sylvestris* are also asymmetrical. Now the two families to which these are generally considered to belong have been placed as intermediate between the *Tanypodinae* and the *Chironominae* in the classification based on adult characters, and also have been shown to be intermediate in the structure of their polytene chromosomes. Their glands, besides being asymmetrical, are intermediate between those of *Tanypus* and *Chironomus* in shape and distribution of nuclei. Thus the internal larval structure can be used to confirm the evidence of external adult relationships.

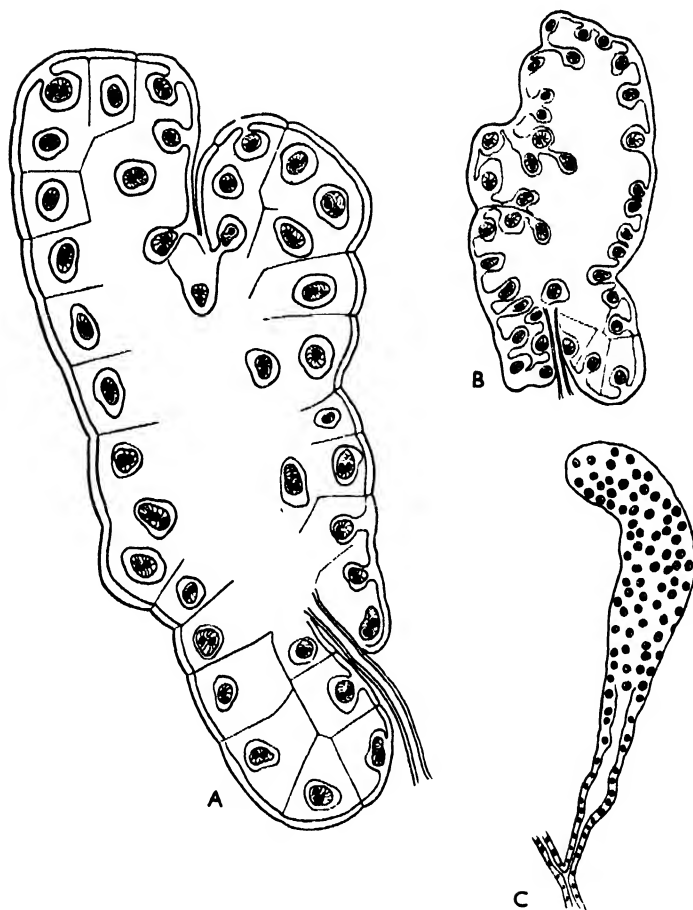


FIG. 3.—Salivary glands of (A) *Chironomus tentans*, (B) *Chironomus dorsalis*, and (C) *Drosophila melanogaster* illustrating development of size. $\times 32$.

SUMMARY.

1. Polytene chromosomes in the Diptera occur not only in salivary glands, Malpighian tubules, and gut cells, but also in non-secretory tissue, *e.g.* nerve cells and anal gills. Vitellophage cells of *Chironomus* contain diplochromosomes.

2. Tissues and organs within a species show different degrees of polyteny, which are correlated with different degrees of uncoiling and nucleic acid charge in the chromosomes. The Chironomidæ, species with the greatest amount of heterochromatin, have the lowest nucleic acid charge on the euchromatin.

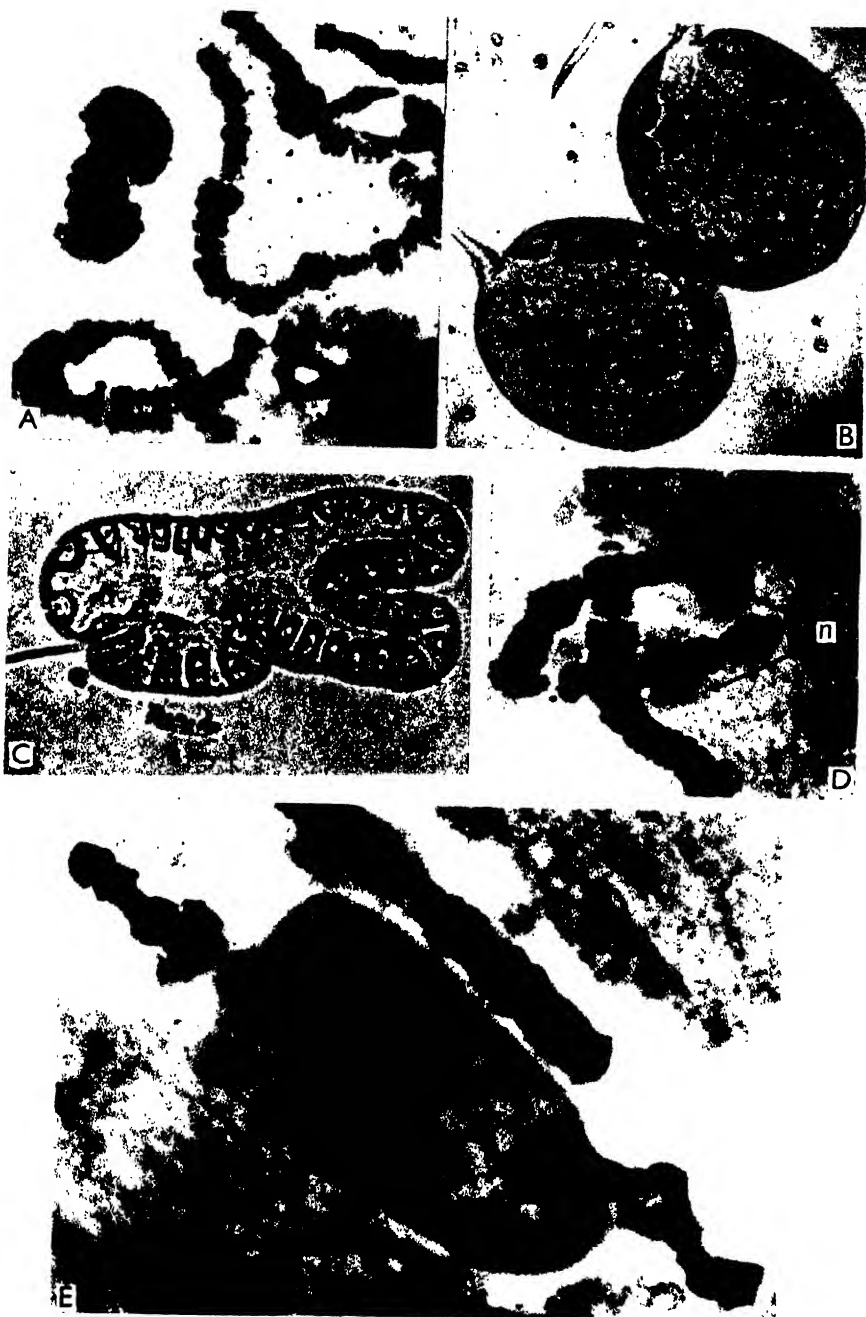
3. There is some evidence of a reversal of the nucleic acid charge of heterochromatin in the Chironominae, and of homology of a heterochromatic region in one species and of a nucleolar organiser in another.

4. Unequal polyteny occurs exceptionally within salivary glands and within nuclei.

5. The organisation of the salivary glands in the Dipteran larva shows evolutionary trends in keeping, on the one hand, with general adult morphology and, on the other hand, with the development of polyteny within the nucleus.

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DESCRIPTION OF PLATE.

- A. Polytene chromosomes from a gland of *Anatopynia varius*. The small "IV" is much thicker than the other chromosomes. $\times 210$.
- B. Salivary gland of *Tanypus* larva. $\times 50$.
- C. Salivary gland of the first instar in *Chironomus tentans*. $\times 50$.
- D. "Nucleoli" (n) on the long and the short "IV" chromosome in *Chironomus* sp. $\times 250$.
- E. Chromosome "IV" with an over-grown nucleolus in *Chironomus pedellus*. $\times 540$.

(Issued separately September 24, 1942.)

XXIV.—The Occurrence of Endodermis in Leguminous Root Nodules and Its Effect upon Nodule Function. By **Helen L. Frazer**, B.Sc., Botany Department, University of Manchester.* *Communicated by Professor J. WALTON, D.Sc. (With Three Text-figures.)*

(MS. received April 11, 1942. Read July 6, 1942.)

INTRODUCTION.

IN recent years considerable progress has been made in the investigation of the biochemistry and physiology of the root nodules of leguminous plants (Wilson, 1940). It now appears desirable that the anatomical structure of the nodule should be re-examined from the standpoint of the functions which the nodule is considered to discharge. Special interest attaches to the distribution within the nodule of endodermis, cork, and any other suberized layers, since on this will depend the facility with which materials may be exchanged between the tissues of the nodule and the environment. There is some confusion in the literature as to the distribution of such suberized tissues within the nodule, but it is clear that rather curious features are presented.

Schneider (1893), as the result of an examination of *Pisum*, *Trifolium*, *Phascolus* and other leguminous plants, reported that the nodules were enclosed by a layer of cork which was said to be joined to the endodermis of the root. He noted lenticel-like structures on the nodules of *Phaseolus vulgaris*, over the vascular strands, and observed that the strands were enclosed in sheaths, though these were not identified by him as endodermal in nature.

Spratt (1919) considered that the outer protective tissues of the nodule were developed from a meristematic layer continuous with the phellogen of the root, and found that their cells might become dead and empty, or in some cases thickened, but she does not make any reference to suberization or to endodermal layers. She observed that the vascular strands in nodules were surrounded by a bundle-sheath.

More precise observations on the distribution of endodermal layers in the nodule were made by later observers. Brenchley and Thornton (1925) and Thornton and Rudolf (1936) reported, for *Vicia Faba* and

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Medicago sativa respectively, the presence of endodermis round the vascular strands of the nodule, and in addition a "lateral" endodermis completely enclosing the central bacterial tissues except for a gap at the apical end of the nodule, where the nodule meristem is situated. Dangeard (1926) reported a similar endodermal system in nodules of a wide range of leguminous plants, including *Pisum*, *Vicia*, *Trifolium*, *Melilotus*, *Phaseolus* and *Lupinus*, but he gave no histological details of the endodermal layers, *i.e.* whether the cells were in the primary, secondary or tertiary condition. McCoy (1929) observed in nodules of *Phaseolus vulgaris*, in addition to endodermis round the bundles, a layer of cells in the nodule cortex which showed structures resembling Casparian strips. Bieberdorf (1938), in a structural study of the nodules of various leguminous plants, makes no reference to endodermis, but reports the presence of corky layers in some nodules.

The purpose of the present paper is to supplement, by means of more detailed observations, the rather fragmentary picture of the distribution of suberized tissue within the nodule that is to be derived from the information given by previous investigators, and also to consider and to study experimentally the effect of these tissues on the exchange of materials between the nodule and the environment. In the morphological section some recapitulation of facts already revealed in previous work is unavoidable; this, however, may be an advantage since it is probable that insufficient attention has been paid to the structural peculiarities of the nodule by those engaged upon the study of its physiological and biochemical characteristics. To a considerable extent the nodules selected for examination in this work are those of plants commonly employed in the investigation of biochemical and physiological aspects of symbiotic nitrogen fixation.

ANATOMICAL OBSERVATIONS.

Nodules of the following leguminous plants were examined: *Pisum sativum* L. (Garden Pea), *Trifolium* sp., *Vicia Faba* L. (Broad Bean), *Phaseolus multiflorus* Willd. (Runner Bean), *Glycine Soja* Sieb. et Zucc., and *Lupinus* sp. (garden hybrid). Some of the plants from which nodules were taken had been grown under field conditions, others in water or sand culture in the greenhouse. The plants grown indoors had all been inoculated with effective strains of nodule bacteria.

Microtome sections were prepared from nodules of different sizes taken from plants in different stages of development, mostly from plants in the flowering or early fruiting stage, the nodules being for the most part preserved in 70 per cent. alcohol. Sections were attached to the

slides by the collodion-clove oil method (Bond, 1931), cleared in eau de Javelle, and either mounted direct in gentian violet-glycerine jelly or stained with sudan III-glycerine or sudan IV and then mounted in clear glycerine jelly. The two sudan stains enable cutinized or suberized membranes to be identified, while the gentian violet is a useful stain for the Casparian strip though it also has some affinity for purely suberized layers. Late on in the investigation a few trials with whole nodules cleared in eau de Javelle and stained with basic fuchsin gave promising results. In such whole nodules the distribution of endodermal layers can readily be studied.

NODULES OF ELONGATED TYPE.

The nodules of pea, clover and broad bean are of this type. As indicated in the Introduction, a double endodermal system has been noted by several workers in examples of the elongated, apically growing type of nodule, comprising the outer, "lateral" endodermis of Brenchley and Thornton (1925), situated to the outside of the vascular system, and individual endodermal sheaths round the vascular traces. In this paper the term "common" (*i.e.* common to the whole vascular system of the nodule) will be used instead of "lateral," since, in respect of spherical nodules, without an organized apex, the latter term is hardly suitable. The common endodermis forms a cylindrical sheath extending from the base of the nodule to within a short distance of its apex. It is interrupted over the apical meristem, while at the base of the nodule it joins on to the endodermis of the root. Fig. 1, A, B, and E, illustrate these features. Thornton and Rudolf (1936) record that in young nodules of lucerne (which are also of the elongated type) the lateral endodermis was in the primary condition, but in older nodules it passed into the secondary stage. In the material examined here no cells of the common endodermis could be identified as definitely primary; they all appeared to be characterized by a complete suberin lamella, and were thus in the secondary condition, even in the case of those endodermal cells near to the apex of still growing nodules. This was true of large branched and small unbranched nodules from pea plants just commencing to flower, and also of the nodules from pea plants only three to four weeks old and from slightly older plants of broad bean. The only material of clover that was examined was from plants in a pre-flowering stage, and here also the common endodermis was entirely in the secondary condition (fig. 1, F). Thus it may be concluded that in these types the nodules are largely enclosed by secondary endodermis during most of their existence.

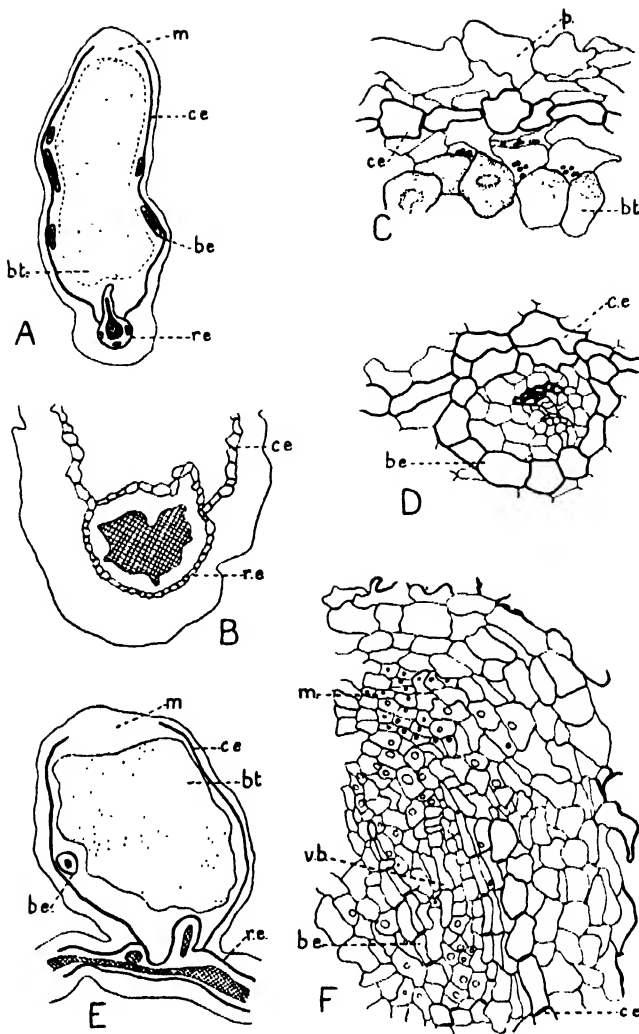


FIG. 1.

A. Longitudinal section of nodule of "Maple" pea (*Pisum sativum*) grown in sand culture, flowering stage, showing common endodermis (secondary), bundle endodermis (primary), root endodermis (secondary), bacterial tissue, and position of meristem. $\times 12$.

B. Basal part of longitudinal section of pea nodule (not median with regard to connecting strand), showing insertion of common endodermis into root endodermis, both secondary. $\times 56$.

C. Outer tissues of nodule of "Gladstone" pea grown in sand culture, early fruiting stage, showing common endodermis, secondary. $\times 180$.

D. Vascular strand seen in transverse section of "Gladstone" pea nodule, showing common endodermis and bundle endodermis, both secondary. $\times 180$.

E. Longitudinal section of clover nodule from soil-grown plants in pre-flowering stage, all endodermis secondary. $\times 32$.

F. Apical part of similar clover nodule in longitudinal section, showing termination of common endodermis (all cells secondary), and a vascular bundle differentiating, partially enclosed by a sheath of primary endodermis. $\times 300$.

c.e., common endodermis; b.e., bundle endodermis; r.e., root endodermis; b.t., bacterial tissue; m., meristem; p., parenchyma; v.b., vascular bundle.

In some larger nodules of pea and broad bean tertiary cellulose lamellæ were observed, internal to the suberin lamella. The tissues outside the common endodermis consisted of parenchymatous cells with intercellular air spaces. In pea and clover nodules this tissue comprised two to five layers of thin-walled cells (fig. 1, C and F); in broad bean the cells were larger with additional cellulose thickening, and the depth of the tissue variable. In the material examined there was no sign of any suberization in tissues external to the common endodermis. The superficial cells of the nodule frequently become disorganized (fig. 1, F) and the broken walls of such cells tended to become stained by gentian violet but not by sudan III.

At the base of the nodule its vascular supply passes out from the root stele, within the area enclosed by the junction of the common endodermis with the root endodermis. The entire vascular system of the nodule was enclosed in sheaths of endodermis in the primary or secondary condition. This bundle endodermis was a direct continuation of the root endodermis, which in all cases examined was in the secondary condition at levels where nodules were borne. In young nodules the endodermis round the nodule vascular strands was primary, with Casparian strips present, but in older nodules it became progressively suberized, a process which started first in the cells nearest to the base, and gradually spread towards the apex. The bundle endodermal sheaths were open in the apical region of the nodule, where the differentiation of the vascular tissues was still in progress (fig. 1, F). The common endodermis in these nodules was either immediately adjacent to the endodermal sheaths round the vascular strands (fig. 1, D) or was separated from these by only a few rows of parenchyma.

NODULES OF THE SPHERICAL TYPE.

The nodules of this type to be studied here were those of runner bean, soya bean and lupin. In these nodules, except in the early stages of development, the growth of the nodule is due to the meristematic activities of a layer extending all round the central bacterial tissue (Fred, Baldwin, and McCoy, 1932; Bieberdorf, 1938). Here too a common suberized endodermis was found in the nodules, external to the vascular system with its endodermal sheaths (fig. 2, A). In nodules of runner bean (*Phaseolus multiflorus*) this suberized layer was situated immediately outside a region of the nodule cortex in which the cells contained numerous calcium oxalate crystals (fig. 2, C). Dangeard (1926) reported the presence of an endodermis in *P. vulgaris* in the same position. In the author's

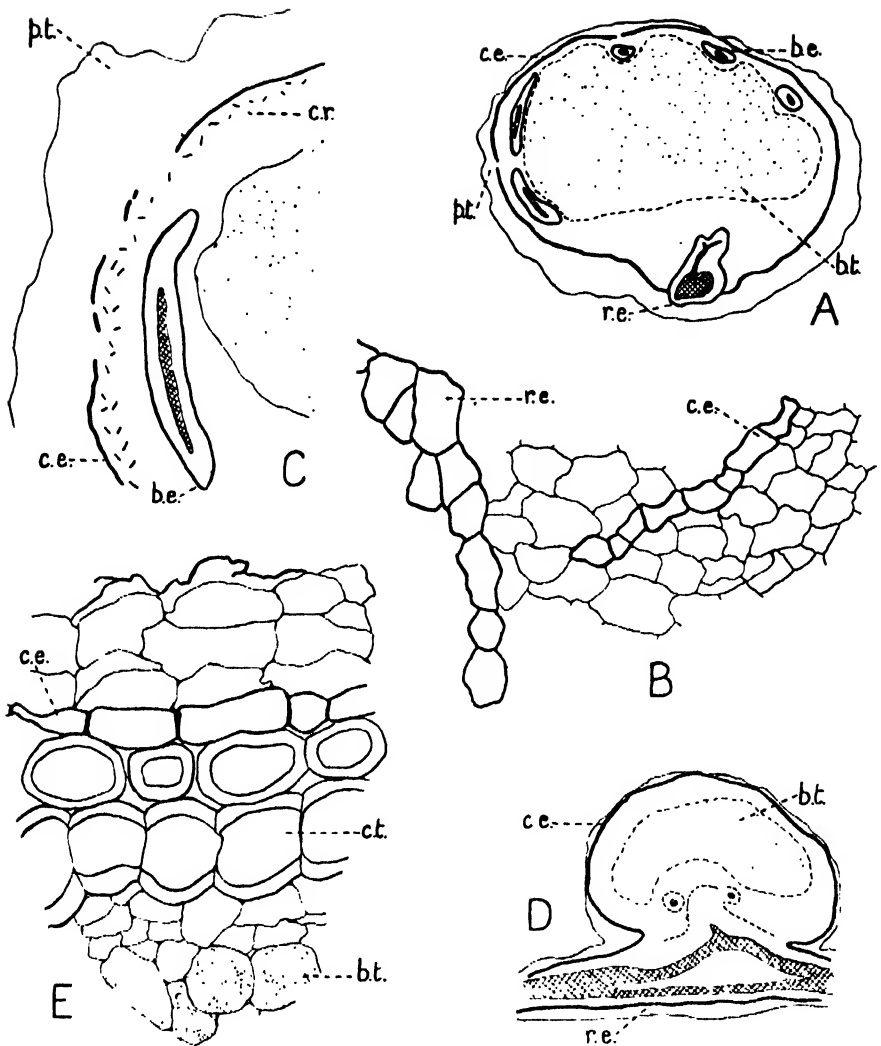


FIG. 2.

A. Section (in plane transverse to root) of nodule of "Scarlet" runner bean (*Phaseolus multiflorus*), from garden, fruiting stage, showing common endodermis with gaps, also bundle and root endodermis, and proliferated tissue, all endodermis secondary. $\times 15$.

B. Basal part of runner bean nodule, showing gap between common endodermis and root endodermis, both secondary. See text. $\times 225$.

C. Outer tissues of runner bean nodule, showing gaps in common endodermis, proliferated tissue, calcium oxalate crystals. $\times 50$.

D. Section (longitudinal to root) of small lupin nodule, from garden-grown plants. $\times 15$.

E. Details of outer tissues of same lupin nodule, showing common endodermis, secondary, and cells with cellulose thickening. $\times 225$.

c.e., common endodermis; b.e., bundle endodermis; r.e., root endodermis; b.t., bacterial tissue; p.t., proliferated tissue; c.r., calcium oxalate crystals; c.t., cells with cellulose thickening.

material of runner bean nodules (from plants bearing young pods) the cells of this layer were suberized on all walls, and occasionally the suberization extended to the walls of neighbouring cells, a feature which was also noted in the vicinity of the endodermis round the vascular bundles of the nodule and of that round the root stele. As in the other types of nodules examined, the radial walls of the layer stained deeply with gentian violet, indicating the presence of Casparian strips below the suberin lamella, and there seems little doubt that the layer is endodermal in nature, and corresponds to the common endodermis of the pea type of nodule. In some of the runner bean nodules this common endodermis was joined to the root endodermis, while in others there was a gap of several cells between the layers (fig. 2, B). Parenchymatous tissue showing no reactions for suberin or lignin covered the nodules outside the common endodermis. This tissue was not of uniform depth. Opposite the vascular bundles of the nodule it was much proliferated, with the inner cells arranged more or less in radial rows. The occurrence of this proliferation made it possible to follow the course of the nodule traces by examination of the surface of whole *Phaseolus* nodules, since the bands of proliferated tissue had a glistening appearance due to the presence of gases trapped between the cells. These areas appear to correspond with those described as lenticels by Schneider (1893). The common endodermis, below the regions of proliferation, showed frequent small gaps of one or two non-suberized cells. It is possible that this structure allows of interchange of materials between the inner tissues of the nodule and the exterior, and compensates for the absence of the apical gap in the common endodermis such as occurs in nodules of the pea type. No cork tissue was detected in these nodules. The proliferated tissue is easily rubbed away, and at points where this had occurred the common endodermis appeared to be the outermost layer (fig. 2, A).

Large nodules of soya bean presented a very similar arrangement of the common endodermis, though this was never observed to be connected to the root endodermis, and usually ended some distance away from it. Young nodules from plants in early stages of development were also examined. According to Bieberdorf (1938), while the young nodule of soya bean grows mainly from the apical region, later on the vascular network closes over the top and further growth is located in a spherical meristem surrounding the bacterial tissue. A conspicuous feature of the soya bean nodule is the presence within the cortex of a ring of thick-walled, lignified cells. These differentiate first in the basal part of the nodule. In nodules from four-week-old plants several stages of endodermal development were noted. In smaller nodules there appeared on

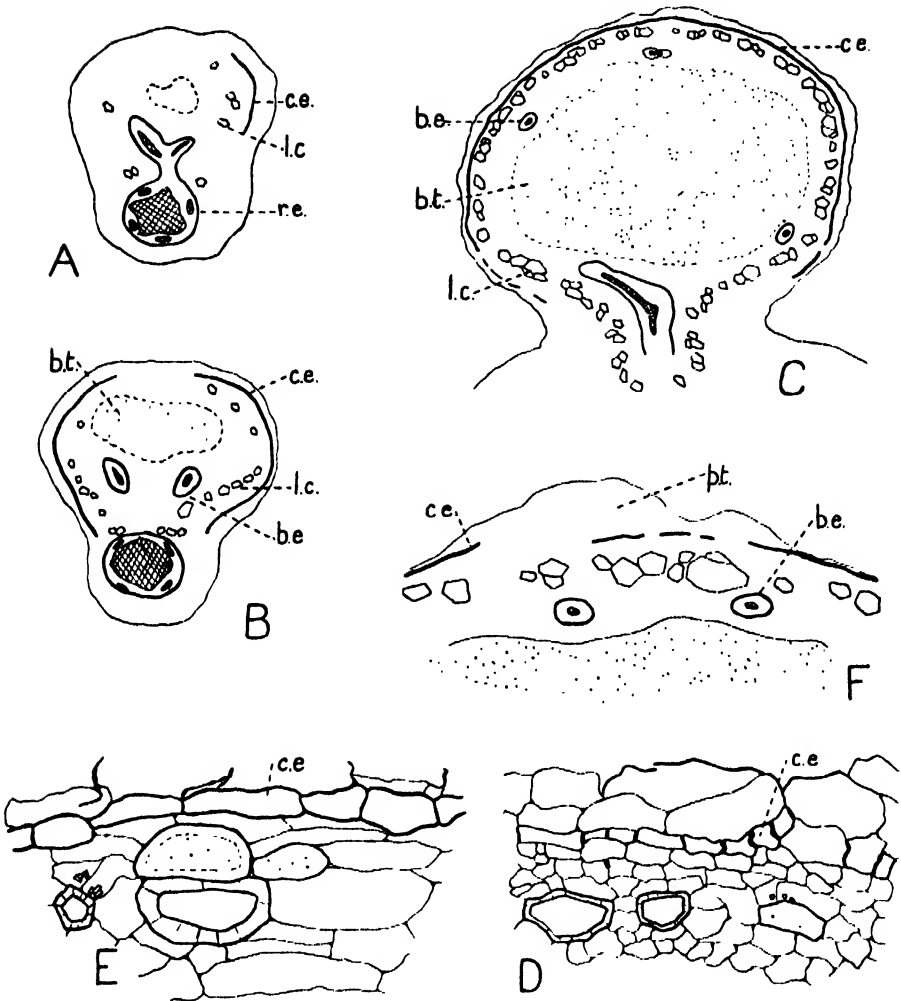


FIG. 3.

A, B, C. Sections of soya bean nodules from plants aged four weeks, in sand culture, showing development of common endodermis, all cells secondary, also bundle endodermis (primary), lignified cells, and bacterial tissue. All $\times 28$.

D. Details of outer tissues of nodule seen in (B), showing common endodermis, secondary. $\times 225$.

E. Details of outer tissues of nodule from ten-week-old soya bean plant, showing common endodermis, secondary. $\times 225$.

F. Part of peripheral tissues of large soya bean nodule, showing proliferated tissue, common endodermis with gaps, and vascular bundles. $\times 50$.

c.e., common endodermis; b.e., bundle endodermis; r.e., root endodermis; l.c., lignified cells; b.t., bacterial tissue; v.b., vascular bundle.

one or both sides of the nodule, outside such lignified elements as had developed, a few cells which from their staining reactions appeared to be secondary endodermal cells (fig. 3, A). In somewhat larger nodules from the same plants this layer of endodermal cells had extended both towards the apex and the base of the nodule (fig. 3, B), while in still larger nodules (diameter 2 mm.) the endodermis, like the vascular tissue and lignified cells, closed over the top of the nodule (fig. 3, C). As in the pea-type of nodule, no cells with Casparian strip only were observed, so that the primary stage, if represented at all, appears to be very short-lived, and in this respect the developmental sequence is not that of a typical endodermis.

With increase in size of soya bean nodules there was considerable tangential stretching of the outer tissues and common endodermis (fig. 3, D, E). The few layers of parenchyma outside the common endodermis were stretched and broken in larger nodules, so that the common endodermis was sometimes on the extreme outside (fig. 3, E). In nodules from plants grown in solution-culture and to a lesser extent in those from sand-culture plants, proliferated parenchyma was found opposite to the vascular bundles, as in the nodules of *Phaseolus*. This tissue was more extensive in soya bean, and its cells were more irregularly arranged. There were gaps in the common endodermis underneath the proliferations, varying in size from a few cells to almost the entire width of the proliferation, and these had the appearance of having arisen by pressure from within and by tangential stretching (fig. 3, F). Proliferations were especially marked in nodules of diameters upwards of 3.5 mm. and appear again to provide a means of communication with the exterior.

In three of the larger soya bean nodules that were examined (diameter 3–5 mm.), the suberized layer, although in the same position as the common endodermis in other nodules, presented rather different features, having become two cells deep in places, with the cells arranged essentially radially. There was, however, no evidence of continued cambial activity. Circumstances have not allowed of a final determination of whether this layer was a development of the original common endodermis, or whether it represented a new cork layer, of the type figured by Bieberdorf (1938) in cowpea, but the writer inclines to the former view.

In all the other large nodules of soya bean that were examined there was no evidence of cork formation, the common endodermis being the only suberized layer outside the lignified cells, even in old nodules whose infected tissue had begun to decay.

Small nodules of *Lupinus* also had a suberized common endodermis

which extended completely round them, and which appeared to be a direct continuation of the root endodermis (fig. 2, D). The endodermal cells showed tertiary cellulose lamellæ. Inside the common endodermis there was a zone of tissue, two to many rows deep, the cells of which had cellulose thickening especially at the corners and on the tangential walls. Further in was small-celled parenchyma in which the vascular bundles were embedded. Outside the common endodermis there was a varying depth of cells with thickened cellulose walls and intercellular spaces (fig. 2, E). In large lupin nodules, however, of the order of 2 cm. diameter, the outermost layers including the common endodermis had disappeared, and a considerable thickness of ordinary parenchyma was present on the outside, apparently arising from divisions of the inner cortical cells of the nodules. In some cases the lupin root bearing a nodule had developed a cork-cambium and cork tissue, but in no case did the cork extend to the nodule.

The vascular systems of nodules of *Phaseolus*, *Glycine*, and *Lupinus* were also enclosed in endodermal sheaths continuous with the root endodermis, although in some of the small lupin nodules the sheath over the early vascular connection only extended towards the root endodermis and had not yet joined it (fig. 2, D). The bundle endodermis was primary or secondary according to the age of the nodule, and became progressively suberized, starting from the base. In all three types, however, occasional cells, especially towards the distal end, retained unsuberized tangential walls, even in old nodules, and these may function as passage cells. There was no definite number or arrangement of these cells.

EXPERIMENTAL OBSERVATIONS.

In view of the probability that a layer of endodermal cells in the secondary condition (*i.e.* with suberin lamella present) offers a serious hindrance to the passage of water and dissolved substances (Priestley and North, 1922) it appears likely that in nodules where the central tissues are largely enclosed in endodermis of this type a certain restriction will be placed on the direct exchange of diffusible materials between the rooting medium and the nodule. Accordingly, such exchange of materials may be expected to occur chiefly through those regions where the common endodermis is incomplete.

Confirmation of these expectations was sought by means of the following experimental procedure. Mature nodules of the types considered in the previous section were freshly excised from living plants along with a piece of the supporting root. The cut ends of the roots

were sealed with melted paraffin wax, and the nodules then immersed in aqueous solutions of various dyes in concentrations that are generally considered to be non-toxic (methylene blue and neutral red .005 per cent.; acid fuchsin .1 per cent.), and also in .5 per cent. tannic acid with subsequent mounting in ferric chloride solution, and in dilute iodine-potassium iodide solution in cases where starch was known to be present in the nodule. After varying periods of time the nodules were thoroughly rinsed in water and the degree of penetration of the bathing solution studied by means of hand sections.

With all nodules it was found that the two basic dyes (methylene blue and neutral red) penetrated into the nodules more rapidly than the acid fuchsin, while iodine penetrated more rapidly than any of the others, though in this case the penetration results in the killing of the cells. Actually, most of the experiments were carried out with the two basic dyes methylene blue and neutral red. With methylene blue it was found that the dehydrase enzymes of the tissues reduced the dye to the leuco-compound when it had penetrated into the infected zone, but as soon as the nodule was cut open the dye was rapidly re-oxidized. The reaction indicates that under the experimental conditions the tissues inside the nodule were experiencing some degree of anaerobiosis.

The course of penetration into all the nodules with apical meristem which were used (pea, broad bean, also sweet pea—*Lathyrus odoratus* L.) was similar. After 24 hours in neutral red or methylene blue solutions the dye was present in all cells of the meristem and in the outer parenchyma of the nodule, but penetration had stopped completely at the common endodermis. In the roots also the dye had only gone as far as the root endodermis. After longer periods of immersion it was found that the dye was spreading through the infected zone from the direction of the apical meristem. This was best seen 48 hours after immersion, by which time the dye had spread about half-way down the nodules. After 72 hours the entire nodule tissues were coloured, but the parenchyma outside the common endodermis was much more deeply stained than the central tissues. It was also noted that as the dyes spread through the central area from the apex, the cells of the nodule cortex internal to the common endodermis became more deeply stained than the infected cells, and that the dye reached the base of the nodule more rapidly through this region than through the infected tissue.

To confirm that the dye reached the central tissues through the apex and not through the common endodermis, nodules of broad bean were decapitated, the cut surfaces sealed with wax, and the nodules then immersed in the dye solutions. In these the tissues inside the common

endodermis were not stained even after eleven days' immersion, by which time the infected area was becoming soft and decayed.

Runner bean and soya bean nodules resembled each other in behaviour, but differed from nodules with apical meristems. Here the dye rapidly penetrated the outer parenchyma until it reached the common endodermis which arrested its progress. In the regions, however, where the proliferated tissue and gaps in the common endodermis occurred, the dye soon passed farther in by way of the non-suberized cells in the gaps. After 24 to 48 hours' immersion of soya bean nodules in dye solution, the dye had penetrated past the lignified elements in the regions opposite the proliferations and had begun to spread tangentially round the nodule cortex as well. With longer periods of immersion the dye continued to spread throughout the nodules, but penetration through the infected zone was relatively slow. Seven days' immersion in neutral red, for example, was required before all the tissues in soya bean nodules 5 mm. in diameter were stained.

Unstained hand sections of the various nodules placed in the dye solutions showed that with free access to the dye the infected tissue became stained less rapidly than the parenchyma round it, but that in less than two hours all became deeply stained. The less rapid penetration into the infected cells may be due to the greater density of the cell contents.

DISCUSSION.

The results of the experiments on solute-penetration support the presumption that the presence of a common endodermis in the secondary or tertiary condition would interfere with direct communication between the interior of the nodule and the soil. It appears that in fully developed nodules of the pea type, direct exchange of materials between the nodule and the rooting medium will be largely restricted to the apical region, where the common endodermis is incomplete, while in the spherical nodules of soya bean and *Phaseolus* the movement of materials seems likely to be concentrated at those points where gaps in the common endodermis occur.

Probably the greatest need for interchange of materials between the nodule and its environment is in connection with the respiratory processes of the nodular tissues and bacteria; in comparison the fixation of nitrogen is a much slower process (Bond, 1941). The results of Bond (*loc. cit.*) and of Asprey and Bond (1941) show that on the dry weight basis the respiration of nodule tissues may be considerably more intense than that of other parts of the plant, though lower figures were obtained by Allison,

Ludwig, Minor, and Hoover (1940). The morphological and experimental observations described in the present paper are in accordance with the indications provided by studies of nodule respiration that the aeration of the nodule is restricted, and that the tissues are in a state of partial anaerobiosis. Thus Allison, Ludwig, Hoover, and Minor (1940) concluded that there is a limited supply of oxygen within the nodule because they found that (*a*) the rate of respiration of nodules maintained in different mixtures of oxygen and nitrogen was promoted as the proportion of oxygen was increased; and (*b*) the respiratory quotient of nodules respiring in air was usually in excess of unity, especially in large nodules, but decreased in atmospheres containing increased proportions of oxygen. (It may be noticed that observations summarized by Wilson (1940) indicate that pure cultures of the nodule bacteria respiring in air usually show a respiratory quotient close to unity.) As a result of studies of another type, Thornton (1931) suggested that it might be the presence of a sub-optimal aeration of the nodule tissues that limits the growth of the nodular bacteria and prevents them from attacking the host cells. Pietz (1938) concluded that the red pigment present in the bacterial tissue of the nodule, by maintaining a favourable oxidation-reduction potential, facilitates bacterial growth despite the restricted aeration.

Another instance of interchange of materials between the nodule and its environment is provided by the excretion of nitrogenous products of fixation from nodules into the rooting medium, demonstrated chiefly through the work of Virtanen (1938). The diffusion of such materials seems likely to be subject to the same restrictions, imposed by the morphological structure of the nodule, as is that of other materials. It is scarcely possible to say at present whether structural differences between nodules of different leguminous plants, or between nodules of the same species growing under different conditions, are in any way responsible for the extremely variable results obtained in experiments relating to this excretion.

The vascular system of the nodule provides another route along which materials may enter or leave the nodule. It is presumably in this way that metabolic products of host origin required by the bacteria arrive in the nodule; it is also conceivable that some oxygen enters the nodule in this way. The presence of the bundle endodermis seems likely, however, to restrict the passage of materials from the vascular strands to the nodule tissues generally. This restriction may not be appreciable in young nodules where the bundle endodermis is still largely primary. In older nodules, however, communication must be restricted more and more to the apical region as the bundle endodermis becomes progressively

suberized from the base, and seems likely to be limited finally to the open ends of the bundles in nodules with apical meristem, or to the non-suberized cells of the bundle endodermis in spherical nodules.

Thornton and Rudolf (1936), in their studies of nodules of lucerne, secured some evidence that in the abnormal nodules developed in the presence of combined nitrogen, the central bacterial tissue fails to secure adequate nourishment because of a restriction placed on diffusion by unusually heavy deposition of suberin in the cells of the lateral (common) endodermis and of thickening of an unspecified nature in the cells of the bundle endodermis. In the types of nodules considered in the present paper it seems that in normal, fully functional nodules also the endodermal layers must exert an appreciable restriction upon the movement of materials within the nodule.

SUMMARY.

1. Nodules of various leguminous plants (*Pisum*, *Vicia*, *Trifolium*, *Phaseolus*, *Glycine*, and *Lupinus*) were examined from the point of view of the distribution within them of endodermal and other suberized layers.

2. All the nodules, except for some large specimens from lupin, were characterized by the presence of a common endodermis in the outer tissues, in addition to individual endodermal sheaths round the vascular strands of the nodule. This is in accordance with statements made by previous investigators in respect of some of the nodule types. In the material examined the cells of the common endodermis appeared to attain the secondary stage (characterized by the presence of a complete suberin lamella) very quickly, and no typically primary cells (with Casparian strips alone) were observed in this layer. The common endodermis was interrupted at the apex in elongated nodules and at points opposite to the vascular strands in the spherical type of nodule.

3. Except for some doubtful instances (in soya bean) no periderm was observed in the nodule material.

4. Experimental observations on the entry of dyes into living nodules indicated that the common endodermis exercised a definite restriction on the direct diffusion of the dyes into the tissues of the nodule. Diffusion occurred mostly through those regions where the endodermal layer was incomplete.

5. The observations indicate that direct communication between the nodule tissues and the exterior must be considerably reduced by the presence of the common endodermis. This seems likely to result especially in a reduced air supply to the inner tissues of the nodule, a state of affairs

which previous investigators, on other evidence, have already suggested to obtain within the nodule.

6. The presence of endodermal sheaths round the vascular strands of the nodule seems likely to exercise some restriction on communication between the nodule and the rest of the plant.

The author wishes to express her thanks to Dr G. Bond who supervised the investigation and gave advice during the preparation of this account of it, and to Dr A. E. W. Boyd who originally commenced the work and prepared many of the microscopical sections. Most of the work was carried out in the Botany Department, University of Glasgow, during the tenure of a Carnegie Research Scholarship.

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XXV.—Growth Stages in the Ammonite *Promicroceras marstonense* Spath. By **Ethel D. Currie**, B.Sc., Ph.D., F.G.S.
Communicated by Professor A. E. TRUEMAN, D.Sc., F.R.S.
 (With Thirteen Text-figures.)

(MS. received June 2, 1942. Read July 6, 1942.)

CONTENTS.

	PAGE		PAGE
I. INTRODUCTION	344	(b) Distance of Siphuncle from Outer Wall of Shell	359
II. MEDIAN WHORL HEIGHT .	347	VI. THICKNESS OF SHELL WALL .	361
III. DEPTH OF IMPRESSED AREA AND MAXIMUM WHORL HEIGHT	353	VII. SEPTA	362
IV. MAXIMUM WHORL BREADTH	356	(a) Thickness of Septa .	362
V. SIPHUNCLE (<i>i.e.</i> SIPHUN- CULAR TUBE)	357	(b) Number of Septa .	363
(a) Diameter of Siphuncle	357	VIII. SUMMARY OF CONCLUSIONS .	365
		IX. REFERENCES TO LITERATURE	366

I. INTRODUCTION.

IT is well known that the dimensions of the ammonite shell conform approximately with the properties of a logarithmic or equiangular spiral. An illuminating account of the logarithmic spiral in its application to the growth of molluscan shells is that given by Sir D'Arcy Thompson (1917, pp. 491–586, and 1942, pp. 748–849). His formula relating the spiral angle of the shell and the ratios of certain linear dimensions must be useful to all interested in shell form in molluscs.

In an amplification of the same subject, Professor Julian Huxley (1932, pp. 149–164) stressed the fundamental fact that organic form is the result of the operation of growth gradients. The logarithmic spiral form must always result in organisms in which growth is accretionary and gnomonic.

Recent work on cephalopods by Professor A. E. Trueman (1941) has thrown light on another aspect of the subject, the relation between body-chamber and air-chambers. It has shown that the form of ammonite shells must have been related to the buoyancy and hence to the mode of life of the ammonite. The idea emerging from this work, that details of shell form in ammonites may have been of adaptational value in

evolution, led Professor Trueman to suggest that I undertake the present inquiry into the rates of change of various dimensions of the ammonite shell during development.

At this point, it may not be inappropriate for me to acknowledge my indebtedness to Professor Trueman for all the help he has given me in this investigation. I am particularly grateful to him for helpful discussions at every stage of the work.

The late S. S. Buckman, who believed in the importance of the dimensions of ammonites in the determination of species, adopted the method (1913, p. viii) of giving dimensions as percentages of the diameter. C. H. Waddington (1929, pp. 180–186), while appreciating the value of ammonite dimensions as aids to their identification, condemned the percentage method. He advocated the plotting of actual measurements because, to mention only one reason, the graphs obtained enable one to see at once whether development is the same or different in different specimens. Buckman's method has been followed by others, and at the present time dimensions of ammonites are mostly given as percentages of the diameter. As noted by Waddington, this tends to obscure the changes that occur in the developing shell. In the present paper actual measurements are plotted.

The rates of change of various dimensions of the ammonite shell can be most easily measured on sections of shells cut through the centre of the protoconch at right angles to the plane of coiling (*i.e.* on transverse sections). Such sections of numerous shells were made in the form of cellulose peels. The method of preparation is that described by Professor J. Walton (1928, p. 571, and 1930, p. 413) for sectioning fossil plants, hydrochloric acid being used for etching and a solution of cellulose acetate and tetrachlorethane (Products of Cellon, Ltd.) for the actual peels. This method was extended by Dr Suzanne Leclercq (1928, p. 24) to the preparation of sections of corals, and later to other invertebrate fossils by my colleague Dr J. Weir who overcame the difficulty of sectioning small specimens by bedding them in plaster blocks, thus obtaining an adequate surface for peeling. The process as applied to fossil corals, ammonites, etc., differs slightly from that introduced by Professor Walton. Etching of the surface of the ammonite section, for example, produces some degree of minute relief of which the peel-section is an impression. In the case of plants, some of the actual plant substance is preserved in the peel-section. In order to obtain the required section, serial sections were made near the centre of each shell, in especially close series near the required position. The well-preserved specimens of *Promicroceras marstonense* Spath from the Marston Marble of Somerset

have been productive of very good sections, which gave opportunities for more detailed study than other specimens examined.

It may be of interest that the cellulose peel-sections were measured immediately they were prepared and were later remeasured for checking

purposes. A certain amount of shrinkage was found to have occurred, on an average 2.5 per cent., but as it had occurred in all directions, there is no material difference between graphs based on the two sets of measurements.

Fig. 1 shows an enlarged outline of a typical median transverse section of *P. marstonense*. Measurements of various dimensions were made at every half-whorl, the maximum number of observations possible for such a section. Measurements of median whorl height (h) and of diameter (D) were made as nearly as possible along the middle line, and it may be noted that in later references to the shell spiral in this paper the spiral in the median longitudinal section is indicated, *not* the spiral of the umbilical suture. Maximum whorl breadth (B) and depth of impressed area (I) were also measured. The siphuncle and shell wall are unusually well preserved in some of these

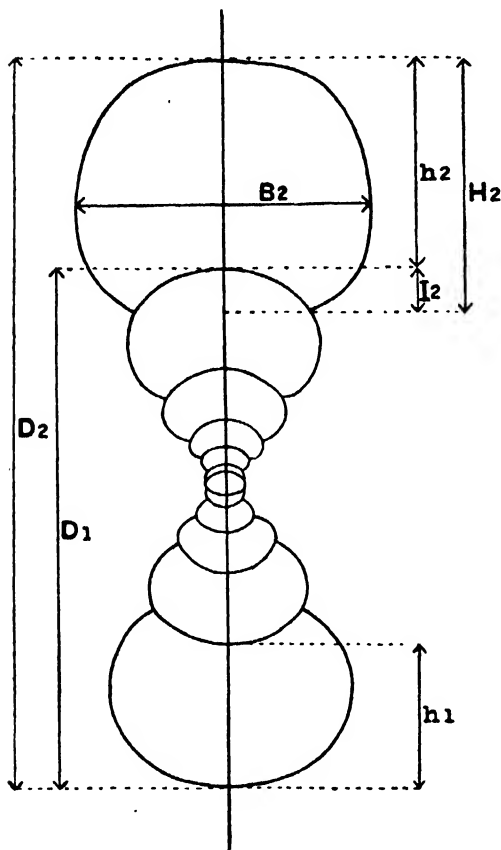


FIG. 1.—Outline drawing of a median transverse section of *Promicroceras marstonense*, $\times 9$. h_1 and h_2 , median whorl heights, and D_1 and D_2 , diameters at interval of a half-whorl. H_2 , maximum whorl height; I_2 , depth of impressed area; B_2 , maximum whorl breadth; all at half-whorl no. 2.

specimens of *P. marstonense* and allowed of fairly accurate measurement. It is convenient to use the term "siphuncle" in this paper in place of the more accurate term "siphuncular tube" (Trueman, 1920, p. 26). Measurements of septum thickness and number of septa were made on median sections cut parallel to the plane of coiling (*i.e.* on longitudinal sections).

In the graphs made to show the rate of increase of various dimensions it was found convenient to plot the dimensions against diameter of shell. Similar graphs were obtained by plotting dimensions against radius of shell.

II. MEDIAN WHORL HEIGHT.

The rate of increase of median whorl height is obviously an important factor in the shaping of the developing shell. It reflects the spiral seen in median longitudinal sections—that is, the spiral formed by the ventral border of the shell.

Although the ammonite shell is essentially based on a logarithmic spiral, measurements in any shell reveal certain departures from a simple equiangular spiral. Measurements of median whorl height in *P. marstonense*, while showing the manner of increase of that dimension, have also given some idea of the deviations in the shell spiral from a true logarithmic spiral.

Median whorl height at every half-whorl was measured on a number of sections of *P. marstonense* with similar results in every case. The graph of median whorl height plotted against diameter (fig. 2) is typical of the species and indicates the general form of the spiral. The graph falls into three parts representing three stages, which are here called α_0 , α_1 , and α_2 stages.

α_0 = The first two half-whorls (to about .75 mm. diameter).

α_1 = The 3rd to the 6th half-whorl (to about 2 mm. diameter).

α_2 = From the 6th half-whorl onwards.

Passing over the first whorl (*i.e.* the α_0 stage) for the moment, it will be seen that beyond that stage, from the 3rd half-whorl onwards, whorl height increases proportionally to the diameter of the shell, but not at a uniform rate. Up to the end of the 6th half-whorl, where the diameter is about 2 mm., the increase is fairly gradual, but after that stage the rate of increase is slightly greater. Similar graphs based on other sections show that the change usually occurs at the 6th half-whorl, but it may occur at the 5th or between the 5th and the 6th. This variation is probably due to the actual position of the measured sections, which may pass near the first septum or up to a half-whorl farther on. The graphs show clearly that the change of growth gradient in the 3rd whorl, although slight, is quite definite.

In one or two of the graphs, such as that of fig. 2, there is a suggestion of a further change of gradient at the 10th half-whorl also.

There is also a change of growth gradient at the end of the first whorl—that is, between the 2nd and 3rd half-whorls. In the graphs of several specimens, the line joining the points that represent the first two half-whorls shows variation in its direction. It may be noted, however, that the gradient of this part of the graph depends largely on the position of the diameter along which each section is cut. This stage (α_0) lasted for

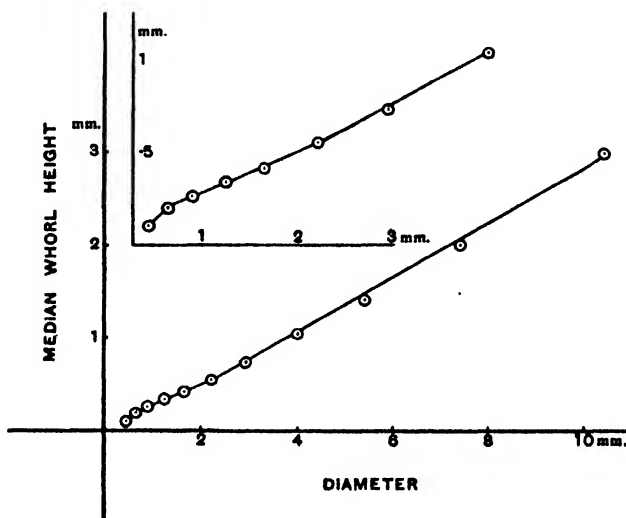


FIG. 2.—Graph showing rate of increase of median whorl height in relation to diameter in *P. marstonense* based on measurements of a median transverse section (P1p) at half-whorl intervals from the 1st half-whorl onwards. The inset shows first part of same graph on a larger scale. Note change of growth gradient at the 6th half-whorl and also between the 2nd and 3rd half-whorls.

so short a time that accurate determination of its character is difficult from such sections.

As indicated by the formula of D'Arcy Thompson (1917, p. 533, and 1942, p. 790) given below, the ratios of successive diameters are directly related to the spiral angle of the shell, in any equiangular spiral.

$$\frac{D_2}{D_1} = e^{\theta \cot \alpha} \quad \text{where} \quad \frac{D_2}{D_1} = \text{ratio of successive diameters.}$$

θ = angle of revolution between D_1 and D_2 .

α = spiral angle.

$$\therefore \cot \alpha = \frac{\log \frac{D_2}{D_1}}{\theta \log e}.$$

It is an interesting property of the logarithmic spiral that in place of $\frac{D_2}{D_1}$, ratios of successive radii $\left(\frac{r_2}{r_1}\right)$ or ratios of successive increments such as median whorl heights $\left(\frac{h_2}{h_1}\right)$ may be substituted.

From the above, it is clear that, in a true logarithmic spiral, ratios of successive median whorl heights and successive diameters (at equal intervals) will be constant, and median whorl height will therefore increase in proportion to the diameter at a uniform rate. The graph of fig. 2, showing two changes in the rate of increase of median whorl

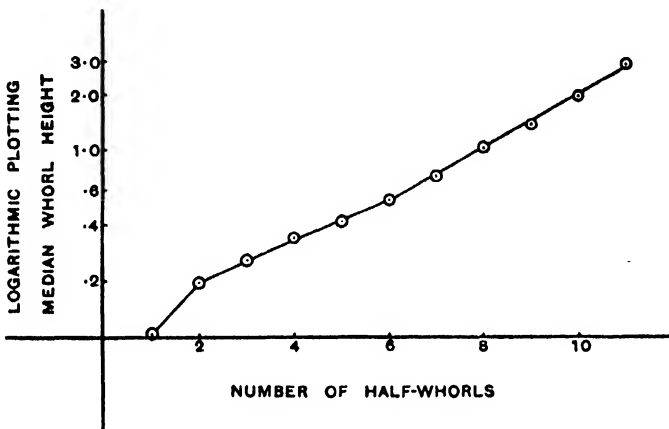


FIG. 3.—Graph showing logarithmic plotting of median whorl height against number of half-whorls based on same section (P1p) as in fig. 2. Note changes of growth gradient at 6th half-whorl and between 2nd and 3rd half-whorls.

height in relation to diameter, indicates that the shell spiral in *P. marstonense* is not a strict equiangular spiral. The three stages, a_0 , a_1 , and a_2 , shown in fig. 2 are emphasised in fig. 3, which shows logarithmic plotting of whorl height against number of half-whorls.

Graphs were also made of median whorl height plotted against diameter at each previous half-whorl, thus relating each median whorl height to a diameter in which it is not included. The graphs based on different specimens are similar to one another and also to the graphs already described (*e.g.* fig. 2) of median whorl heights in relation to diameters at the same half-whorl. They show a break between the 2nd and 3rd half-whorls and a change of gradient at the 6th half-whorl, thus illustrating once again the a_0 , a_1 , and a_2 stages referred to above. They also show an indication of a slight change of gradient at the 10th half-whorl.

It is apparent that calculations of the spiral angle based on ratios of median whorl heights will not in these circumstances give results identical with those derived from ratios of diameters, since the spiral at any stage encloses an earlier stage with a different curvature. In other words, the median whorl height ratio of stage α_2 would imply a certain spiral angle only if the same ratio had been maintained earlier; the formation of the spiral corresponding to that ratio is precluded by its being based upon an earlier stage of different character. It is clear that ratios of diameters will more correctly reflect the form of the spiral than ratios of whorl heights.

The spiral angles α_1 and α_2 for the two stages (α_1 and α_2) have been calculated from ratios of median whorl heights and from corresponding diameters for several specimens; the results are as listed below.

Number of Section.	Based on Ratios of Median Whorl Heights.		Based on Ratios of Corresponding Diameters.	
	α_1	α_2	α_1	α_2
P1 <i>p</i>	85° 26'	83° 51'	84° 36'	84° 23'
P10 <i>d</i>	85° 33'	83° 47'	84° 53'	84° 33'
P8 <i>e</i>	85° 53'	83° 33'	84° 45'	84° 12'
P15 <i>e</i>	85° 17'	83° 26'	84° 43'	84° 8'
P13 <i>b</i>	85° 47'	83° 33'	85° 4'	84° 5'
P1 <i>p</i> (2)	85° 22'	83° 43'	84° 32'	84° 11'
P11 <i>b</i>	85° 21'	84° 10'	84° 47'	84° 35'
Average values	85° 31'	83° 43'	84° 45'	84° 18'

It should be noted that the above values were calculated from points falling exactly on the lines of each graph. Actually, there is a slight fluctuation in the ratios of both successive whorl heights and successive diameters at every half-whorl. It is apparent, then, that the graphs relating whorl height and diameter show the general trends at what are here called the α_1 and α_2 stages of development, and it is of interest that the values of the spiral angle at corresponding stages are approximately constant for the species.

It will be seen that the angles calculated on median whorl heights show a greater difference at the two stages than the angles deduced from corresponding diameters. Obviously, the difference between α_1 and α_2 based on diameter ratios must depend on the change in median whorl height. An increase of median whorl height ratio must produce a much smaller change in the ratio of diameters, since diameters include the unaltered earlier whorls. In this connection it is interesting to compare fig. 7 (*b*), showing logarithmic plotting of diameters at every half-whorl, with fig. 3, showing logarithmic plotting of median whorl heights at

half-whorl intervals. The graph of diameters shows the three stages, α_0 , α_1 , and α_2 , but its changes of gradient are smaller than those in the graph of whorl heights.

Values of the spiral angle in the first whorl (α_0 stage) have been calculated for several specimens and are given below, but the data on which they are based is not regarded as wholly satisfactory. It is clear, however, that the change from the α_0 to the α_1 stage between the 2nd and 3rd half-whorls is a bigger change than the later change about the 6th half-whorl, although the latter has been more fully discussed.

Number of Section.	Based on Ratios of Median Whorl Heights.	Based on Ratios of Corresponding Diameters.
	α_0	α_0
P1 ϕ	78° 27'	83° 29'
P1 ϕ (2)	81 43	81 37
P10d	83 6	82 54
P8e	82 39	82 46
P11b	83 44	83 0
P15e	83 3	83 2
P13b	85 3	83 24

The section P1 ϕ is cut along a diameter that passes near the first septum.

As mentioned previously, there is actually some fluctuation in the ratios of successive whorl heights at every half-whorl, which is accompanied by fluctuations in the ratios of corresponding diameters. In the case of ratios of diameters, the fluctuations are naturally smaller. Fig. 4 shows whorl height ratios (solid line) and diameter ratios (broken line) at every half-whorl for two different specimens: the two sets of fluctuations for each specimen are superposed and together illustrate one or two points of interest.

It may be recalled that, for a spiral with a uniform spiral angle, such graphs would both be horizontal straight lines, the ratios of both median whorl heights and diameters being constant. It will be seen that the graph of diameter ratios differs only slightly from a straight line. Actually it consists of two parts, the first with a slight downward trend, the second with a slight upward trend, and the two parts meet about the 6th half-whorl, the point at which the α_1 - α_2 change in spiral angle occurs. The graph of median whorl height ratios shows greater deviation from a straight line and falls into three parts, which clearly represent the α_0 , α_1 , and α_2 stages. In the α_1 stage in this graph the line runs below the graph of diameter ratios and in the α_2 stage it runs above it, the crossing-point of the two graphs being at or near the 6th half-whorl.

It is evident that this (the 6th half-whorl) marks a most important change in the growth ratios of the spiral. After that change, the median whorl height increases at a greater rate than previously. Meanwhile the ratios of the corresponding diameters increase very slightly, so that for a period the ventral spiral becomes very nearly a true logarithmic spiral.

The graphs also cross somewhere between the 2nd and 3rd half-whorls, indicating the change from the α_0 to the α_1 stage.

A change of spiral angle (based on whorl height ratios) was noted by D'Arcy Thompson (1917, p. 540, and 1942, p. 797) in a specimen recorded

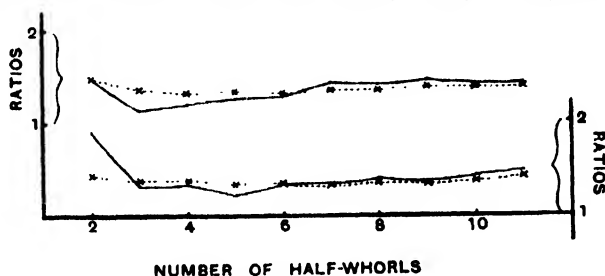


FIG. 4.—Graphs based on two median transverse sections of *P. marstonense* (P1p and P10d) to show (a) ratios of whorl height to preceding whorl height at every half-whorl (solid line) and (b) ratios of diameter to preceding diameter at every half-whorl (broken line). (a) and (b) are superposed for each specimen. To save space, the two pairs of graphs are drawn close together with their vertical scales marked at opposite sides of the figure.

as *Arcestes tornatus*, according to series of measurements given by A. H. Grabau (1872 or 1882). It is not clear in which of Grabau's two papers the measurements are given and it has not been possible to obtain, in Britain, a copy of either of them. This change is described by D'Arcy Thompson as an increase of the ratios and consequently a diminution of the angle α from the centre of the coil outwards. Professor Huxley (1932, p. 161) also noted that in some ammonites "the ratio of the diameters of successive whorls does not remain constant as in the true logarithmic spiral, but increases progressively."

In *Promicroceras marstonense*, on the other hand, the change in spiral angle about the 6th half-whorl is not gradual but takes place with apparent abruptness. D'Arcy Thompson's series of values of the spiral angle of Grabau's specimen suggests to the writer that they may represent two or more changes of spiral angle at different stages. The writer has found that in certain other ammonites measured (*Emileia*) there are two changes of gradient (in addition to a change at an early stage) in the graphs of median whorl height plotted against diameter, indicating corresponding changes of spiral angle. In other shells measured

(*Sphaeroceras*) there are three such changes. These changes in growth gradient are not gradual but occur at certain stages of development, and they sometimes represent an increase and sometimes a decrease of spiral angle. Details of these and other ammonites will be published later.

In examining the deviations of the shell spiral from a true logarithmic spiral, one must consider the protoconch, for the ammonite shell is a spiral built around this initial chamber. The distinction was emphasised by Naumann (1848 and 1864) and Grabau (1872) in introducing the term "conchospiral." It may be noted that D'Arcy Thompson (1917, p. 531, and 1942, p. 788) was in error in calling attention to an apparent confusion in the use of the term "protoconch." The "space" to which he referred is actually the protoconch, and the initial form of the spiral does not depend on the "little bulbous or hemispherical chamber" (siphonal cæcum) but on the form of the protoconch itself, to which the first whorl is moulded. Unfortunately, the measurement of diameters of a protoconch even in an accurate median section introduces special difficulties, and it has not been possible to calculate ratios which can be regarded as satisfactory for the present purpose.

The general results of the measurements of the ventral spiral therefore indicate that the ammonite shell may change its mode of coiling once or oftener during development, and it seems likely that the shell spiral at any stage must deviate from a true logarithmic spiral by an amount depending on the earlier stages of the shell's development. The spiral angle of the initial whorls (based on diameter ratios) thus depends on the ratio of median whorl heights and on the form of the protoconch. The spiral angle (based on diameter ratios) of the succeeding whorls (a_1 stage) must be determined by both the ratio of median whorl heights and by the form of the preceding whorls. After the addition of several whorls with a constant median whorl height ratio, the spiral angle based on diameter ratios may be expected to become practically indistinguishable from that calculated from the ratio of median whorl heights (fig. 4).

III. DEPTH OF IMPRESSED AREA AND MAXIMUM WHORL HEIGHT.

The state of preservation of some shells, together with slight asymmetry in some sections, made measurement of the depth of impressed area rather difficult. The absence of perfect symmetry is probably partly due to slight obliquity of the sections. Graphs of depth of impressed area plotted against diameter, based on several specimens, show some variation, no doubt due largely to inaccuracies of measurement; but the main features of these graphs (e.g. fig. 5) are constant for the species.

The depth of the impressed area in *P. marstonense* decreases sharply from the 1st half-whorl and is least of all in the 2nd half-whorl. From this minimum point the depth of impressed area increases rapidly and proportionally to the diameter up to the 8th half-whorl (sometimes to the 7th). The increase in the 5th whorl appears to be irregular, but it is certainly slight. In the first half of the 6th whorl there is probably a slight rise in the gradient of the graph. This, however, is observable only in the largest specimens, those over 10 mm. in diameter.

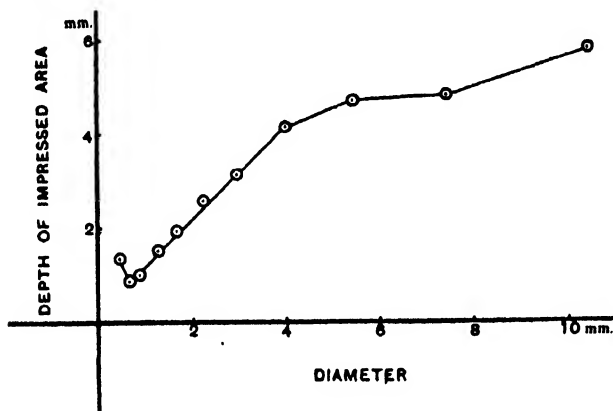


FIG. 5.—Graph showing the relation of depth of impressed area to diameter of shell in *P. marstonense* based on measurements of a section (P1p) at half-whorl intervals. Note minimum point at 2nd half-whorl and marked change in growth gradient at the 8th half-whorl.

The maximum whorl height at any stage is obviously the sum of the median whorl height and the depth of impressed area. Graphs of maximum whorl height plotted against diameter based on several specimens show similar results. The graph of fig. 6 shows that maximum whorl height increases rapidly in proportion to the diameter from the 2nd whorl, *i.e.* the 3rd half-whorl onwards. The rate of increase in the first two half-whorls is less rapid. In this graph (fig. 6) the change of growth gradient occurs just before the 3rd half-whorl measurement; in similar graphs based on other specimens the change of gradient occurs at the 2nd half-whorl, or between the 2nd and 3rd half-whorls. This variation no doubt depends on the position of the diameter along which the section is cut.

Direct measurement of the umbilical spiral would be extremely difficult, but distances between umbilical sutures and diameters of the umbilicus can be calculated from measurements of whorl height and depth of impressed area. Fig. 7 (a), showing logarithmic plotting of diameter

of umbilicus at half-whorl intervals which reflects the umbilical spiral, is typical for *P. marstonense*. The graph approximates fairly closely to a straight line from about the 5th half-whorl onwards. The form of the graph between the 2nd and 5th half-whorls is not perfectly clear. It may rise in a curve, but certainly it rises rapidly though not at a uniform rate. In any case, as the diameter of the umbilicus does not increase at a uniform rate, the umbilical spiral is not a true logarithmic spiral. This is especially true of the early whorls (2nd–5th); from about the 5th half-whorl onwards there is closer approximation to an equiangular

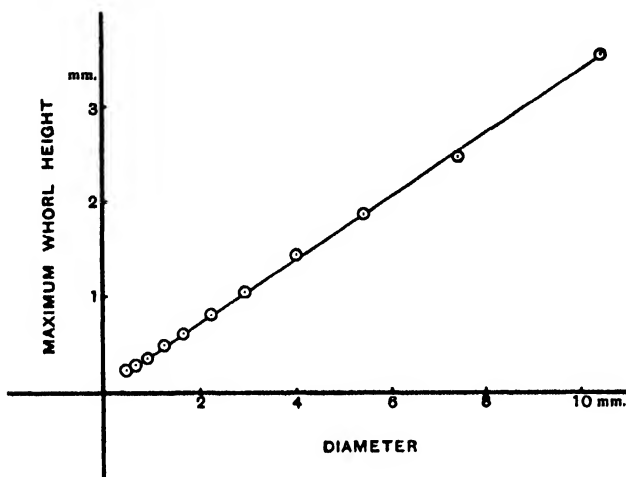


FIG. 6.—Graph showing relation of maximum whorl height to diameter of shell in *P. marstonense* based on measurements of a section (P1p) at every half-whorl. Note change of growth gradient between 2nd and 3rd half-whorls.

spiral. In addition to a marked increase in the spiral angle between the 4th and 5th half-whorls, there is also a slight change of gradient between the 8th and 9th half-whorls, representing a slight decrease of spiral angle. The latter change probably has some relation to the reduction in the rate of increase of impressed area at the 8th half-whorl (see fig. 5).

It is interesting to compare this graph (fig. 7 (a)) with the graph beside it (fig. 7 (b)), showing logarithmic plotting of diameters of the shell spiral (ventral) at half-whorl intervals in the same specimen. The graph of fig. 7 (b) shows even closer approximation to a straight line, although actually there is a slight change of gradient at the 6th half-whorl and a more marked one between the 2nd and 3rd half-whorls, illustrating the three stages, a_0 , a_1 , and a_2 , which are so much more clearly defined in graphs of median whorl height (*e.g.* fig. 3).

The two graphs (fig. 7 (a) and (b)) give contrasting pictures of the

umbilical spiral and the ventral spiral respectively. It is clear that the course of the umbilical spiral is dependent to a great extent on the amount of overlap of each whorl on the preceding one, *i.e.* on the depth of impressed area. Divergence of the two graphs would therefore indicate a tendency of the shell to become involute, whereas closer approximation of the graphs would indicate that the shell is becoming more evolute. It seems, then, from comparison of the two graphs (fig. 7 (a) and (b)), that

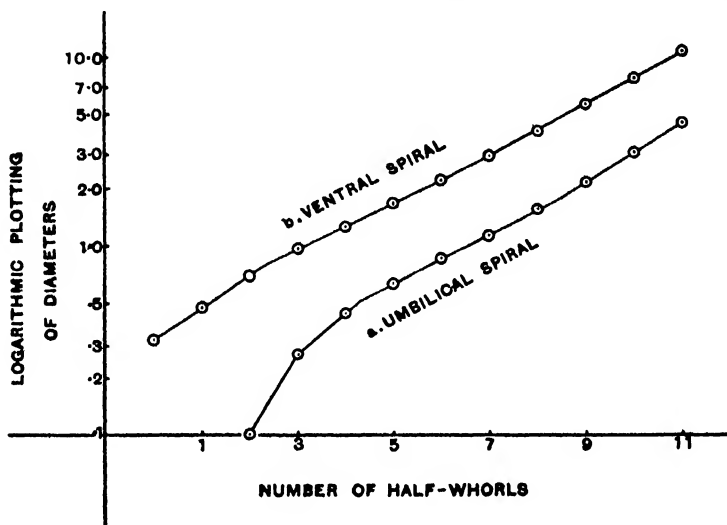


FIG. 7.—Graphs showing logarithmic plotting of (a) diameter of umbilicus and (b) diameter of shell at half-whorl intervals, based on the same median transverse section (P10d). Note changes of gradient in (a) between 4th and 5th half-whorl and between the 8th and 9th; and in (b) between the 2nd and 3rd half-whorls and at the 6th half-whorl.

in the initial stages of *P. marstonense* the shell is involute, and becomes less involute (or more evolute) up to about the 5th half-whorl. From that point the amount of overlap remains approximately the same until the latest stages, when the shell tends to become slightly more evolute.

IV. MAXIMUM WHORL BREADTH.

Measurement of whorl breadth at every half-whorl in several specimens showed that the mode of increase of maximum whorl breadth is constant for the species. The graph of maximum whorl breadth plotted against diameter (fig. 8) is typical, and shows two changes in the growth gradient and the points at which they occur.

There is no appreciable increase in breadth in the first whorl (up to 7–8 mm. diameter), which has the same breadth as the protoconch.

After that, whorl breadth increases rapidly, in proportion to the diameter, up to a point in the 4th whorl (usually at 8th half-whorl measurement; sometimes between the 7th and 8th), where the diameter is usually between 4.0 and 4.4 mm. Beyond that stage whorl breadth is still proportional to diameter, but the rate of increase is less rapid.

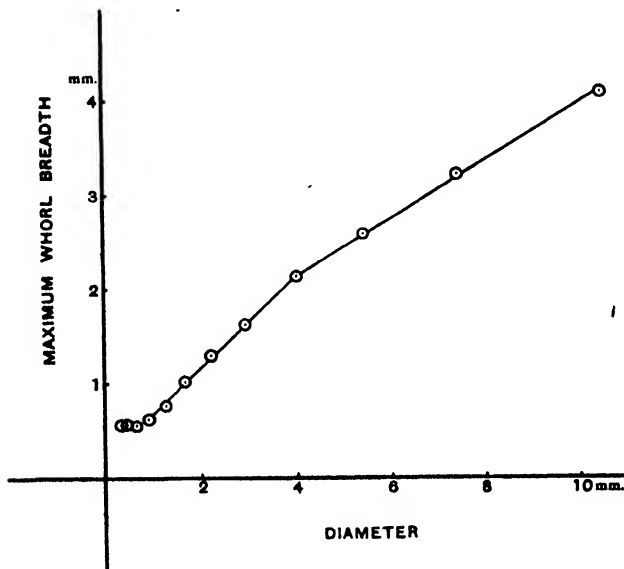


FIG. 8.—Graph showing rate of increase of maximum whorl breadth in relation to diameter of shell in *P. marstonense* based on measurements of a section (Pip) at half-whorl intervals from the protoconch onwards. The part of the graph between the 3rd and 8th half-whorls is represented by the equation $B = .5D + .15$ and the part beyond the 8th half-whorl by the equation $B = .3D + .91$.

It was found, from the graph of fig. 8, that the equation $B = .5D + .15$ represents the line of the graph between the 3rd and 8th half-whorls (D =diameter and B =maximum whorl breadth). The same line of the graphs for other specimens gave the following equations: $B = .5D + .2$, $B = .52D + .16$, $B = .48D + .19$.

Equations obtained for the line of the graph beyond the 8th half-whorl are as follows: $B = .3D + .91$ (fig. 8), $B = .29D + 1.0$, $B = .285D + 1.1$, $B = .28D + 1.2$.

V. SIPHUNCLE (*i.e.* SIPHUNCULAR TUBE).

(a) *Diameter of Siphuncle*.—In most specimens of *P. marstonense* available for sectioning the siphuncle is preserved only in the first 6–7 half-whorls, up to a stage where the diameter of the shell is usually

between 2 and 3 mm. In the 1st half-whorl, where the shell diameter is usually about .5 mm., the siphuncle diameter is approximately .05 mm.

Diameter of the siphuncle was measured in a series of specimens and graphs were made, plotting siphuncle diameter against diameter of shell. From these, it seems that the siphuncle diameter increases uniformly in proportion to the diameter of the shell up to the 6th or 7th half-whorl, where the shell diameter is almost 3 mm. The short line graph of fig. 9

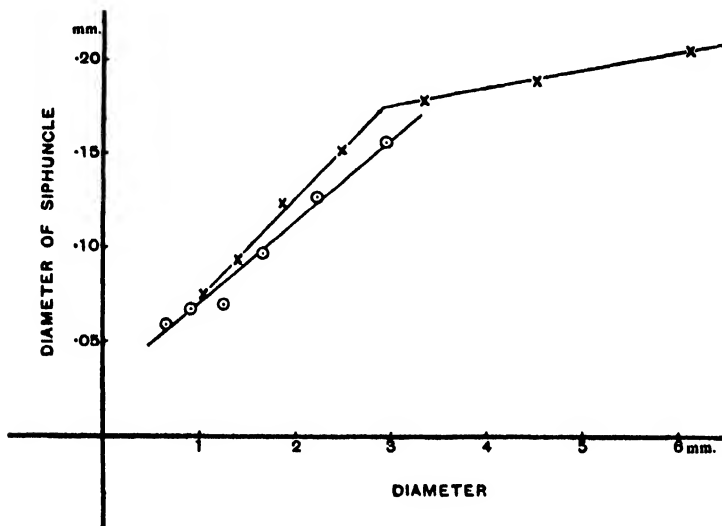


FIG. 9.—Graphs showing rate of increase of diameter of siphuncle in relation to diameter of shell based on two median transverse sections of *P. marstonense*, one (P11p: open circles) with siphuncle preserved from the 2nd to the 7th half-whorl and the other (P11b: crosses) with siphuncle preserved from the 3rd to the 9th half-whorl.

is typical of most of the specimens measured. Only one specimen (P15e) differs from those described in showing a sudden large increase in the siphuncle diameter in the 5th half-whorl.

Only two specimens among those sectioned have the siphuncle preserved beyond the 7th half-whorl. One of these (P11b) is of special interest on account of its being the only specimen with siphuncle continuously preserved as late as the 9th half-whorl. At this stage (9th half-whorl) the diameter of the shell is about 6.5 mm. In this specimen the siphuncle diameter in the early stages increases in proportion to the shell diameter, and at approximately the same rate as in other specimens. Beyond the 6th half-whorl the siphuncle diameter still increases in proportion to the shell diameter, but the rate of increase is very much reduced (fig. 9). In the other specimen (P7f) the change in

the growth gradient seems to occur between the 7th and 8th half-whorls, instead of between the 6th and 7th as in P11b.

Professor Trueman (1941, p. 355) has shown that in *Dactylioceras commune* the siphuncle diameter is uniformly proportional to the diameter of the shell up to a diameter of 5 mm., which led one to expect some similar relation between siphuncle diameter and shell diameter in *P. marstonense*.

(b) *Distance of Siphuncle from Outer Wall of Shell*.—The distance of the siphuncle from the wall in specimens of *P. marstonense* is variable. Its distance from the ventral margin varies and at the same time its course deviates laterally to some extent from the median longitudinal plane. Sections parallel to the plane of coiling rarely show the complete siphuncle; even when the siphuncle is continuous in the section, a slight deviation of the siphuncle or the section from the median plane will give a wrong impression of the diameter of the siphuncle. Sections at right angles to the plane of coiling, *i.e.* transverse sections, are therefore the most useful for showing both the course of the siphuncle and its full diameter.

In the 1st half-whorl the distance of the siphuncle from the outer wall appears to vary between .011 and .038 mm. Values of this dimension for several specimens are listed below. As in previous cases, variation may depend on the actual position of the section. Although this distance is variable, it has been found that the distance of the *centre* of the siphuncle from the wall is a fairly constant proportion of the whorl height (as shown in table below).

Number of Section.	Distance of Siphuncle from Wall in 1st Half-Whorl.	Half Diameter of Siphuncle in 1st Half-Whorl.	Distance of Centre of Siphuncle from Wall in 1st Half-Whorl.	Proportion of Whorl Height.
P1 <i>φ</i>	.011 mm.	.032 mm.	.043 mm.	39.2 per cent.
P1 <i>φ</i> (2)	.032 "	.032 "	.065 "	30.8 "
P13 <i>b</i>	.032 "	.027 "	.059 "	34.9 "
P8 <i>e</i>	.038 "	.027 "	.065 "	36.0 "
P15 <i>e</i>	.027 "	.03 "	.057 "	35.4 "
P10 <i>c</i>	.038 "	.027 "	.065 "	38.1 "

It will be seen that in the 1st half-whorl the centre of the siphuncle is 30–40 per cent. of the whorl height from the outer wall of the shell. Possibly the differences may be due to an actual reduction in the diameter of the siphuncle in the space between successive septa, a feature sometimes seen in median longitudinal sections.

Graphs of the distance of the siphuncle from the wall plotted against diameter (fig. 10) show that, although the maximum distance from the

wall is variable in amount, the maximum point occurs always at the same stage in the development of the shell, *i.e.* at the 2nd or 3rd half-whorl (usually at the 3rd half-whorl), where the diameter of the shell is about 1 mm. Furthermore, as shown in the appended list, the position of the

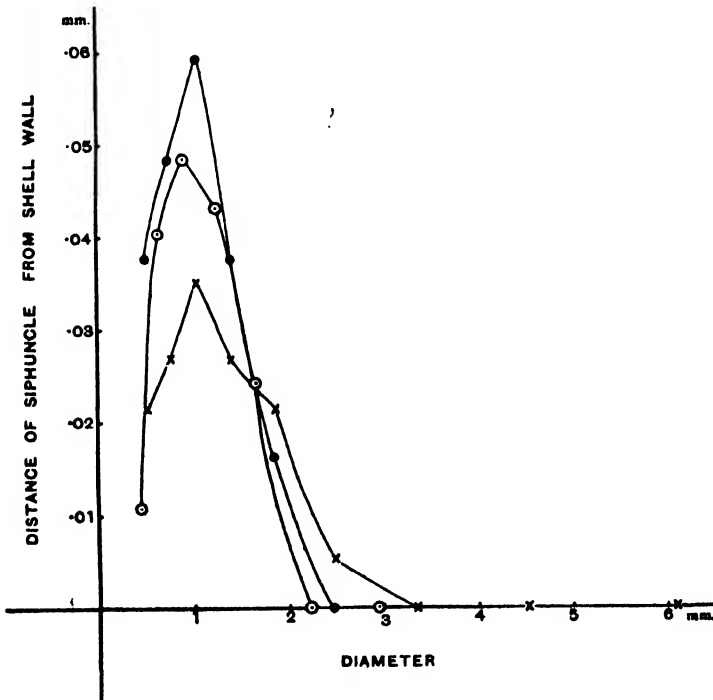


FIG. 10.—Graphs showing distance of the siphuncle from the shell wall in its course from the 1st half-whorl onwards, based on measurements of three median transverse sections (P8e: black circles, P1p: open circles, and P11b: crosses) at every half-whorl. Note that the maximum distance occurs always at the same stage, the 3rd half-whorl, and that the siphuncle becomes external, *i.e.* in contact with the wall, at the 6th or 7th half-whorl.

centre of the siphuncle at the maximum distance from the wall bears an approximately constant relation to the whorl height.

Number of Section.	Maximum Distance of Siphuncle from Wall.	Half of Siphuncle Diameter.	Distance of Centre of Siphuncle from Wall.	Proportion of Whorl Height.
P1p	·049 mm.	·034 mm.	·082 mm.	30·5 per cent.
P1p(2)	·038 "	·036 "	·074 "	24·7 "
P13b	·038 "	·035 "	·073 "	27·0 "
P8e	·059 "	·032 "	·092 "	30·6 "
P11b	·035 "	·038 "	·073 "	25·1 "
P15e	·038 "	·041 "	·078 "	27·0 "
P10e	·059 "	·032 "	·092 "	31·6 "

The above figures show a range of variation in the maximum distance of the siphuncle from the wall. They also show that the maximum distance of the centre of the siphuncle from the wall is between 24 per cent. and 32 per cent. of the whorl height.

From the maximum distance about the 3rd half-whorl the siphuncle approaches the wall until it is touching it, at the 6th or 7th half-whorl. The siphuncle continues in contact with the wall from the 7th half-whorl onwards.

In 1933, Dr L. F. Spath (1933, p. 431) had observed little variation in the position of the siphuncle within a species. At a later date, however, he (1938, pp. 8-10) concluded that there is no regularity in the rate of growth and position of the siphuncle, and his remarks lead one to believe that he has found this variability even within a species.

The results given above for *P. marstonense* admittedly show some variability in the distance of the siphuncle from the wall, but the variability occurs within certain limits. The results also show a definite regularity in the rate of growth and position of the siphuncle as follows:—

1. The siphuncle diameter in *P. marstonense* is proportional to the diameter of the shell. Professor Trueman (1941, p. 355) has already found this to be true for *Dactylioceras commune*, and the writer has recently found the same rule in some specimens of *Emileia*.

2. In *P. marstonense* the maximum distance of the siphuncle from the wall occurs at a certain stage of development (3rd or 2nd half-whorl).

3. In *P. marstonense* the siphuncle becomes external (*i.e.* in contact with the outer wall) at a definite stage (6th or 7th half-whorl).

Dr Spath's table (1938, p. 9) of some data of different species includes the stage at which the siphuncle becomes external. The stage certainly varies in the different species, but it is noteworthy that in the three specimens of *Promicroceras planicosta* listed the siphuncle becomes external at approximately the same stage, *i.e.* 2-2½ whorls.

VI. THICKNESS OF SHELL WALL.

This paper is not concerned with the structure of the shell of *P. marstonense*, but it is necessary to note its general appearance. It appears that the structure of the shell wall is essentially the same throughout, from the protoconch to the outermost whorls, and it consists of a central prismatic layer of Aragonite (according to Miegen's Test and an S.G. test) with a thin dark-coloured outer layer, and a similar inner layer the nature of which has not yet been ascertained. In the earliest whorls the prismatic layer is very thin and the outer and inner

marginal layers are prominent. As the shell increases in thickness during development the central layer becomes thicker, and the marginal layers, which do not actually change in thickness, appear proportionately thinner. In the outermost and thickest part of the shell the marginal layers appear almost negligible.

The thickness of the shell (*i.e.* central prismatic layer and marginal layers) at every half-whorl was measured on a number of specimens. Allowing for slight inaccuracies of measurement, especially of the thin walls of the protoconch and earliest whorls, uniform results were obtained.

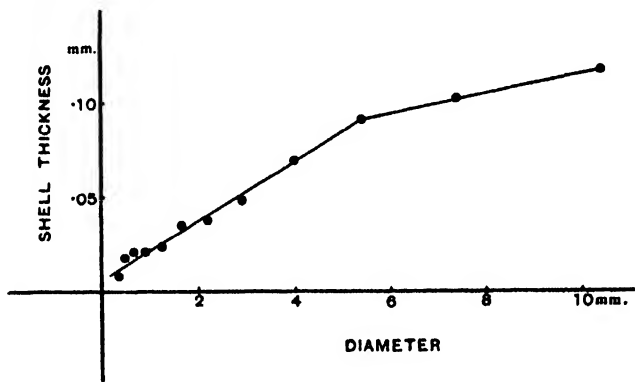


FIG. 11.—Graph showing rate of increase of shell thickness in relation to diameter of shell in *P. marstonense* based on measurements of a section (P1*p*) at intervals of a half-whorl from the protoconch onwards. Note change of gradient at the 9th half-whorl.

The shell thickness of the protoconch is usually 0.008–0.01 mm., although in one specimen it appears to be as much as 0.016 mm. Fig. 11 is typical of the graphs of shell thickness plotted against diameter which show that the shell thickness increases fairly rapidly in proportion to the diameter from the protoconch to the 9th half-whorl. Beyond the 9th half-whorl the rate of increase, which is still proportional to the diameter, is very gradual.

The above results regarding shell thickness were obtained from specimens of *P. marstonense* about 10 mm. in diameter, and do not necessarily apply to the later stages of larger specimens with stronger ribbing.

VII. SEPTA.

(a) *Thickness of Septa.*—The structure of the septa in *P. marstonense* appears to be similar to that of the shell wall. They are extremely thin however.

On a section (P6*b*), cut parallel to the plane of coiling, thicknesses of septa were measured at every half-whorl. It was found that there is no

appreciable increase of thickness of septa in the first three half-whorls where the thickness measures about .005 mm.; but the difficulty of measuring such a very thin structure makes it impossible to be certain. From this stage onwards, septum thickness increases fairly rapidly in proportion to the diameter of the shell (fig. 12).

It has previously been shown (Trueman, 1941, p. 353) that septum thickness in *P. marstonense* and also in *Dactylioceras commune* is, in general, proportional to the diameter.

(b) *Number of Septa*.—The number of septa in every quarter-whorl was counted in four sections of *P. marstonense* cut parallel to the plane

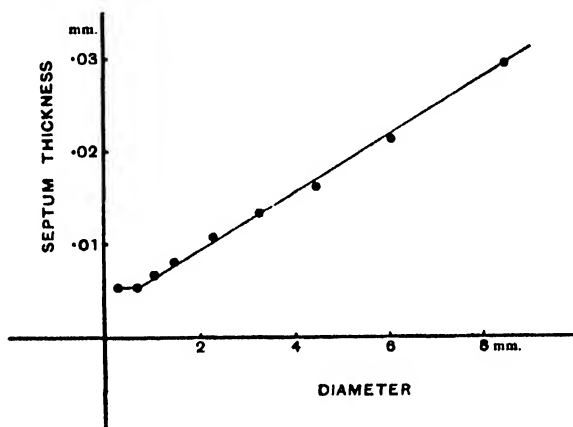


FIG. 12.—Graph showing rate of increase of septum thickness in relation to diameter of shell in *P. marstonense* based on measurements of a longitudinal section (P66). Change of growth gradient occurs at the 3rd half-whorl.

of coiling. Several counts were made on each specimen with slightly different placing of the diameters, and although the results vary slightly in detail similar features are shown in each case.

The arrangement of the septa appears at first sight to be very irregular, some septa being set close together and others quite far apart. The counts of septa for two of the sections (Trueman Coll., Nos. 3 and 5), however, show quite striking agreement both as regards number and spacing of the septa, and the graphs based on these sections, of number of septa at every quarter-whorl (fig. 13), emphasise this similarity. Another section counted (Trueman Coll., No. XI) is incomplete, but so far as it goes it is in agreement with Nos. 3 and 5. The fourth section available (P307) shows less striking agreement with the others, in that the maximum in the 2nd and 3rd whorls is lower and begins a quarter of half of a whorl earlier. Nevertheless, the agreement among these

four sections suggests the possibility that the sequence of the septa in a species may be characterised by a certain rhythm which may be the result of seasonal growth changes. The rate of growth of the individual shell depending on food supply and other circumstances (Spath, 1919, p. 70) would no doubt modify the rhythm of septum formation and possibly the diameter attained at particular stages.

The graphs of number of septa plotted against number of whorls (fig. 13) show a maximum number of septa between $1\frac{1}{2}$ and $2\frac{1}{2}$ whorls. This must correspond either to a period of more frequent septum formation

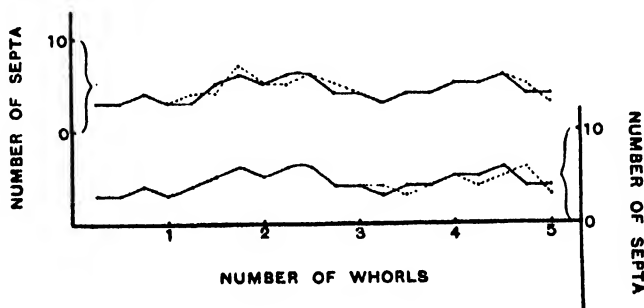


FIG. 13.—Graphs showing number of septa in every quarter-whorl of *P. marstonense* based on two median longitudinal sections (Trueman Coll. Nos. 3 and 5). The solid and broken lines represent alternative counts. Note maximum number of septa between $1\frac{1}{2}$ and $2\frac{1}{2}$ whorls and also in the 5th whorl.

or to a period of slower shell growth, septum formation being at the same rate: the latter seems the more likely explanation. If this is assumed to be the case, and bearing in mind that in *P. marstonense* the body-chamber is rather more than half of a whorl in length (Trueman, 1941, p. 367), it appears that shell growth may have been slow when the complete shell was between 2+ and 3+ whorls in size (1.5–2.5 mm. in diameter). The graphs suggest a change to a more rapid rate of growth beyond that stage, and it seems significant that this change in growth rate begins in the 6th half-whorl and is presumably related to the stage at which the α_1 – α_2 change in spiral angle occurs. It is probable that this marks the commencement of a new season's growth. There appears to be another and shorter period of slow growth in the 5th whorl also, when the complete shell was 5+ to $5\frac{1}{4}$ + whorls in length.

It is stated in Eastman's edition of Zittel's *Text-Book of Palaeontology* (1900, p. 510, and 1913, p. 591) that septa "vary exceedingly in number among different species and also at different ages of the same individual; but they are tolerably constant within the limits of one and the same species if specimens of the same age are compared. They follow one

another in regular succession, but, as observed by Hyatt, the intervals are relatively greater in the young, more constant in the adult, and then markedly decrease in the oldest stages of development." The first part of this statement was repeated in the more recent German edition of Zittel's *Text-Book* (1924, p. 514). Dr L. F. Spath (1919, p. 67, and also 1933, p. 440, and 1938, p. 10), while noting the above statement, drew attention to great variation in the number of septa not only in different species but also in individuals of one species. The results obtained in the study of *P. marstonense* suggest that one may expect to find some degree of uniformity in the spacing of septa within a species if attention is paid to the effects of differing growth rates.

Some evidence of seasonal growth stages in *Dactylioceras*, *Promicroceras*, and *Arnioceras* has been described by Professor Trueman (1941, pp. 345-346 and 367-368), and it is possible that the spacing of the septa reflects similar features.

VIII. SUMMARY OF CONCLUSIONS.

1. It has been shown that the ammonite shell approximates closely to a logarithmic spiral, but that in some species changes of spiral angle occur once or oftener during development. In *P. marstonense* a change of spiral angle occurs about the 6th half-whorl and also between the 2nd and 3rd half-whorls.

2. The angle of the spiral changes with a change in the rate of increase of the radius of the spiral (as shown by changes in the ratio of successive whorl heights). Estimates of spiral angles based on ratios of whorl heights and on ratios of diameters are not the same, the difference between the values of two spiral angles based on whorl heights being greater than the difference between estimates based on corresponding diameters. It has been shown that ratios of successive diameters (and consequently spiral angles based upon them) depart only slightly from the constant state of a strict logarithmic spiral.

3. In *P. marstonense* the umbilical spiral, like the median spiral, is not a strict equiangular spiral, but changes its spiral angle at two points, the first between the 4th and 5th half-whorls, the other between the 8th and 9th.

4. In *P. marstonense* maximum whorl breadth increases in a manner that is constant for the species. There is no increase of whorl breadth in the first whorl, which has the same breadth as the protoconch. Thereafter, maximum whorl breadth increases in proportion to the diameter, with a change of growth gradient at the 8th half-whorl.

5. In *P. marstonense* the diameter of the siphuncle increases in proportion to the diameter of the shell, but with a change in the growth gradient either between the 6th and 7th half-whorls or between the 7th and 8th half-whorls.

6. The distance of the siphuncle from the wall in *P. marstonense* is variable to some extent; but the siphuncle does show some regularity in its course. In the 1st half-whorl the centre of the siphuncle is found to be between 30 and 40 per cent. of the whorl height from the outer wall of the shell. The maximum distance of the siphuncle from the wall occurs always at the same stage, about the 3rd half-whorl, and at this stage the centre of the siphuncle is 24-32 per cent. of the whorl height from the outer wall of the shell. The siphuncle becomes external—that is, in contact with the wall—at the 6th or 7th half-whorl.

7. The thickness of the shell wall in *P. marstonense* was found to be proportional to the diameter of the shell, the rate of increase being much reduced at the 9th half-whorl.

8. Septum thickness in *P. marstonense* was found to be uniformly proportional to the diameter of the shell from the 3rd half-whorl onwards. No appreciable increase of septum thickness was apparent in the first three half-whorls.

9. The sections available suggest that the septa in *P. marstonense* show some indication of a rhythmic spacing which is regarded as reflecting the growth stages of the shell.

10. The conclusions listed above show how the form of the ammonite shell is the result of several growth gradients operating simultaneously.

11. The above conclusions also suggest that the 6th half-whorl was an important stage in the development of the shell of *P. marstonense*. Important changes also took place between the 2nd and 3rd half-whorls.

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(Issued separately October 2, 1942.)

XXVI.—The Induction of Pregnancy in the Golden Hamster during the Breeding Pause. By **O. Peczenik**, M.D., Department of Zoology, University of Glasgow. *Communicated by* Professor **EDWARD HINDLE**, F.R.S.

(MS. received June 12, 1942. Read July 6, 1942.)

IN 1930 ovulation in ferrets outside the breeding season was obtained by Hill and Parkes (1930) by means of gonadotrophic extracts. Later, similar results were obtained in sheep and thus it became possible to induce pregnancy outside the normal breeding season (Cole and Miller, 1933), and its practical importance was investigated by Parkes and Hammond (1940). Similar experiments on the golden hamster, *Cricetus auratus*, are described below, as this animal has proved to be a most interesting subject for the study of sex hormones (Klein, 1937 and 1938; Peczenik, 1942).

The animals used are descended from a litter found in 1930 in Syria and first bred at the Hebrew University at Jerusalem. In the following year two pairs, the offspring of the original litter, were presented by Dr S. Adler to Professor Hindle, who succeeded in raising a stock of hamsters which have been distributed to various laboratories and forms the basis of all observations in this country in which these animals have been used (Bruce and Hindle, 1934).

In this stock of animals two notable changes have occurred:

(1) During the breeding season of 1941 several females were found either to have been sterile during the whole life-cycle or to have ceased breeding long before the upper age-limit for breeding (10 months) had been reached. It was found that a certain percentage of these sterile individuals was susceptible to influence by gonadotrophin or oestrogen, and subsequently produced litters.

(2) Bruce and Hindle (1934) found that the majority of females did not breed from October to March; Deanesly (1938), although confirming this observation, found that the cyclic changes proceed regularly, even during the winter months, and suggested that any loss in fertility during the winter was due to the male. However, recent investigations which I

have carried out with the same stock during 1941-42 indicate a change in the behaviour of the females resulting in the appearance of a breeding pause, and 16 out of 19 became anæstrous about the middle of October. The 3 exceptions showed irregular cycles, and in addition had protracted œstrus, resembling that seen in old mares (Hammond, 1938) and hibernating bats (Courrier, 1925). Metœstrus, as distinctive from the results obtained during the breeding season, was not recognizable in the vaginal smears.

From December onwards there was a gradual increase in the number of individuals in which œstrus occurred. Regular and continuous 4-day cycles, however, did not begin until the end of February or the beginning of March—that is, immediately before, or at the beginning of, the breeding season. Certain unpublished observations by Hindle support the supposition that the two changes mentioned might be, in part, the result of genetic factors.

During the breeding pause females, both over and under the upper age-limit of breeding, were treated with either gonadotrophic extracts or œstrogen. These included animals which had been fertile as well as those which had been sterile during the previous breeding season. The results, together with those obtained during the 1941 breeding season (Peczenik, 1942), are summarized in the following table:—

RESULTS OF TREATING STERILE AND SENILE FEMALE HAMSTERS WITH GONADOTROPHIN OR ŒSTROGEN, INDICATED BY THE PRODUCTION OR NON-PRODUCTION OF LITTERS, DURING THE BREEDING SEASON 1941, AND THE BREEDING PAUSE 1941-42; AND ALSO THE RESULTS OF TREATING NORMAL FEMALES DURING THE BREEDING PAUSE.

Type of Female.	Treated with Gonadotrophin.		Treated with Œstrogen.	
	Positive.	Negative.	Positive.	Negative.
(a) Sterile (or senile) females during breeding season	7	4	4	3
(b) Sterile (or senile) females during breeding pause	0	8	0	1
(c) Normal females during breeding pause	4	1	0	5

EXPERIMENTS WITH GONADOTROPHIC HORMONE.

Experiments on 13 females were begun at the end of January 1942. Seven of the animals were 13 to 21 months old. Three of these had been sterile during the 1941 breeding season and were treated with

chorionic gonadotrophin; 2 responded, but the 3rd remained sterile. The remaining 6 females were 8 to 9 months old.

Eight of the 13 animals received, on two consecutive days, 6 mg. per day of AP118B, a mixed horse anterior pituitary extract, for which I am indebted to the kindness of Dr A. S. Parkes. On the third day, 15 I.U. (100 γ) of chorionic gonadotrophin (Pregnyl) were injected. AP118B, which is rich in follicle stimulating hormone, was combined with chorionic gonadotrophin which has a predominantly luteinizing action, in order to obtain a maximum number of ovulations (Pincus, 1939; Fevold and Hisaw, 1934).

The other 5 females received, on three consecutive days, 6 mg. of AP118B per day, without the addition of chorionic gonadotrophin. Solutions of the extracts were injected subcutaneously in the back.

Twenty-four hours after the last injection of AP118B or of chorionic gonadotrophin respectively, the females were mated for fourteen days with 5- to 8-month-old males. Eight of the males had received 6 mg. of AP118B on ten consecutive days preceding the mating. Tests on animals selected at random had shown that several testes contained no spermatozoa during the breeding pause. Examination carried out after mating showed ample spermatogenesis in the injected males and spermatozoa in the tubuli.

The hormone provoked œstrus in all the injected females. As regards fertilization, the effect depended not only on the age of the animal but also on whether it had been sterile or fertile during the preceding breeding season. During the breeding season, 4 out of 6 sterile individuals over 10 months old and 3 out of 5 sterile females under 10 months were fertilized, either during the œstrus provoked by the treatment or in one of the spontaneous œstrus following in 4-day cycles. During the breeding pause, on the other hand, 6 out of the 7 females over 10 months old remained infertile; it being immaterial whether they had received both gonadotrophins or only AP118B, and whether their males had been treated or not. The seventh female, injected with AP118B alone and paired with a treated male, was fertilized but aborted. Of the 6 animals under 10 months old, 4 littered. Contrary to the results of mating during the breeding season, when fertilization followed either provoked or spontaneous œstrus, during the breeding pause fertilization did not occur in the provoked œstrus. The latter was generally succeeded by 4-day cycles and the animals were fertilized during the second spontaneous œstrus. The litters of the 3 younger females survived, while the young of the fourth mother, 9½ months old, died within the first four days after birth. This last female had received both extracts. Of the 3 other

mothers, 2 had been injected with AP118B and urine extract, the third with AP118B alone. Of the two females in this group (under 10 months old) which were refractory to treatment, one had been sterile during the 1941 breeding season and was not influenced by chorionic gonadotrophin. The fathers of two of the surviving litters had been treated; the fathers of the third litter, which survived, and of the litter which succumbed, had not been treated. Of the two refractory young females, the sterile one had been mated with a treated male, the other with an untreated male.

EXPERIMENTS WITH ŒSTROGEN.

Steinach, Staheli, and Gruter (1934) found that a single injection of œstrogen induces œstrus, ovulation, and fertility in sterile cows presumably by regulating the function of the anterior pituitary.

During the 1941 breeding season 7 sterile female hamsters, $3\frac{1}{2}$ to 22 months old, were treated by this method, each receiving a single subcutaneous injection of 40 γ of stilbœstrol; they were paired sixteen to twenty-one days after the injection. A similar experiment was carried out on 21st January with 6 females, 5 of which had been proved to be fertile during the previous breeding season; the 6th was then still immature. On the eighteenth day after the injection the females were paired with males under 10 months old, each of which had received 6 mg. of AP118B per day on the ten preceding days. Whilst in the series carried out during the breeding season 4 out of the 7 sterile individuals, from $3\frac{1}{2}$ to 22 months old, produced litters, not one of the animals injected during the breeding pause had a litter.

Early in the present breeding season (1942) one of the animals treated with gonadotrophin and one injected with stilbœstrol have produced litters, although both animals had, in the interim, passed the upper age-limit of breeding and the treatment had not been repeated. Both litters, however, died within the first four days after birth. It should be noted, in this connexion, that during the 1941 breeding season the litters of females more than 10 months old, which had been provoked into fertility either by chorionic gonadotrophin or by stilbœstrol, also died within the first four days after birth. Only a single offspring survived of a female, which had been reinjected with stilbœstrol after the death of its first litter.* Sterile young females, on the other hand, produced viable and fertile litters after the treatment.

* In three experiments carried out with gonadotrophin during the 1942 breeding season, repetition of the treatment after one to three months had elapsed also induced survival of the litters of females over 10 months old.

DISCUSSION.

The experiments have shown that it is possible, by injections of gonadotrophic extracts, to cause normal golden hamster females to litter outside of the breeding season. Apparently the administration of a small quantity of mixed horse anterior pituitary is alone sufficient, and its combination with a principally luteinizing hormone seems to be irrelevant. The combination of both gonadotrophins was used in the expectation that an increase of the number of shed ova during the provoked œstrus might increase the chance of fertilization. However, fertilization never occurred during the provoked œstrus but only during a following spontaneous œstrus.

In contrast with the results obtained during the breeding season, it was not found possible to cause sterile young animals or senile individuals to litter. The experiments show no certain grounds upon which this distinction rests, but two possibilities present themselves:

(1) It may be that the sterility in the stock is made continually more resistant to influence by hormones because the factors which may cause or even compel sterility become continually more operative with every increase in the number of the generations bred in captivity. In the recent breeding season (until the beginning of July) only 4 of 9 sterile individuals treated with both gonadotrophins, and 3 out of 5 sterile individuals injected with chorionic gonadotrophin, became pregnant. A further individual injected with AP118B alone, remained sterile. The present breeding season is, however, not yet sufficiently advanced for the results to be comparable with those obtained in 1941.

(2) Referring to the second possibility, it may be that the difference between the results obtained during the breeding time and the non-breeding time is causally connected with the seasonal rhythm. The second assumption is supported by the noted result that it was impossible to bridge over the breeding pause by means of stilbœstrol, but during the breeding season it was possible to overcome sterility by the injection of this œstrogen. This assumption is in agreement also with observations of the behaviour of various other mammals during the breeding pause (Hill and Parkes, 1930; Parkes and Hammond, 1940; Marshall, 1942).

It is with pleasure that I acknowledge my best thanks to Professor Edward Hindle for his continued and helpful assistance in these studies. I am very much obliged to Dr A. S. Parkes for ample supplies of AP118B and valuable data on its activity in rats, and to Dr A. N. Macbeth, Organon Laboratories, for Pregnyl.

SUMMARY.

In the golden hamster (*Cricetus auratus*) a breeding pause was observed during the winter 1941-42.

In two-thirds of the experiments it was possible to bridge over the breeding pause by treatment of the female with anterior pituitary gonadotrophin, or by a combination of the latter with chorionic gonadotrophin.

Whilst it was possible to get litters from sterile and also from senile females treated with gonadotrophin during the breeding season, this effect could not be produced outside the breeding time, contrary to the results obtained in normal animals.

Single injections of a moderate amount of œstrogen, which induced fertility in sterile females during the breeding time, did not influence females during their breeding pause.

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374 *Induction of Pregnancy in Golden Hamster during Breeding Pause*

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XXVII.—The Larval Maxillary Glands of *Euphausia superba* Dana.

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INTRODUCTION.

THE adult antennal glands of Euphausiids have been described by Chun (1896), Zimmer (1913), Raab (1915), and Cannon and Manton (1927). In the course of an investigation into the development of these organs, undertaken by me at the suggestion of Professor H. Graham Cannon, it was discovered that they arise quite late in the life-history, whilst earlier larvæ were found to possess typical maxillary glands. This finding was not unexpected, for an inverse relationship between antennal and maxillary segmental excretory organs has been noted in the development of many Crustacea.

It is the purpose of the present paper to describe the structure of these maxillary glands in the larvæ of *Euphausia superba*, in which their occurrence has not been previously reported. Since embryos, nauplii and metanauplii of *Euphausia superba* were not available, and attempts to distinguish the rudiments of maxillary glands in the earliest larval stages of related species failed, the origin and early development of the glands could not be determined.

Changes in the external features of Euphausiids during their larval development have been described by many authors from Claus (1863) to Fraser (1936); the latter gives a complete list of the literature on this subject. Work on their early embryology and internal development is, however, meagre. The changes that can be observed through the cuticle of entire animals were described by Claus (1863); Sars (1897) discussed the early segmentation stages, and Cannon and Manton (1927) noted that in the nauplii of Euphausiids there was no trace of an antennal gland. The only extensive work on Euphausian embryology that I could find is Taube's (1909, 1915) account of the development of *Nyctiphanes norvegicus* and *Boreophausia raschii* as far as the first furcilia. Taube makes no mention of maxillary glands, but they probably escaped his

attention, since in furcilia stages of *Nyctiphanes couchii* I found maxillary glands apparently identical with those of *Euphausia superba*.

All references to larval stages in this communication are based on the nomenclature of Fraser (1936).

ACKNOWLEDGMENTS.

I wish to thank Professor H. Graham Cannon for his advice and criticism. My thanks are also due to the Discovery Committee for much material. I am indebted to Professor W. M. Tattersall and Dr Robert Gurney for some specimens, and to Miss Leak whose slides of *Nyctiphanes couchii* I examined.

MATERIALS AND METHODS.

The bulk of the material used formed part of the large collection made by the Discovery Expedition (1925-1927). It had been fixed in alcohol and showed some shrinkage, so that the finer details of cell structure were, at times, difficult to determine. The method of double embedding was used for all specimens. After they had been treated with a weak solution of celloidin for at least seven days, the smaller specimens were embedded in paraffin wax and the larger ones in a mixture of wax with ceresin. Transverse, parasagittal and oblique sections, 4, 6 or 8 μ in thickness, were cut and stained either with Mallory's triple stain or with Weigert's hæmatoxylin, followed by Orange G—Erythrosin as a counterstain. The reconstruction was aided by wax models, the technique employed being that recommended by J. R. Norman (1922).

DESCRIPTION.

The following description refers to the structure of the maxillary glands as seen in the fourth furcilia, the most convenient stage for investigation. They are, however, fully formed in the first calyptopis, but from the fifth furcilia onwards, in which stage the antennal glands seem to be functional, the maxillary glands show signs of progressive degeneration, until, in late adolescents, no traces of them remain.

The maxillary glands of the fourth furcilia are situated in the ventro-lateral regions of the body, and each consists of an end sac, sphincter valve, efferent duct and exit-tube (fig. 1). The end sac is a pear-shaped structure occupying a lateral lobe of the body which projects between the edge of reflexion of the carapace and the bases of the second maxilla and the first thoracic limb. The thin wall of the end sac (w.e.s.) is composed of four cells only, an unusually small number, situated on a basement

membrane, and exhibiting the vacuolated cytoplasm and lozenge-shaped nuclei characteristic of such organs. The boundaries of the cells are indeterminate; the nuclei bulge towards the cavity of the end sac, and the vacuoles contain globules of a granular precipitate. The wall of the end sac is attached by fine fibrils of connective tissue to the lateral ectoderm and by coarse tendinous strands to the dorso-lateral and ventro-medial

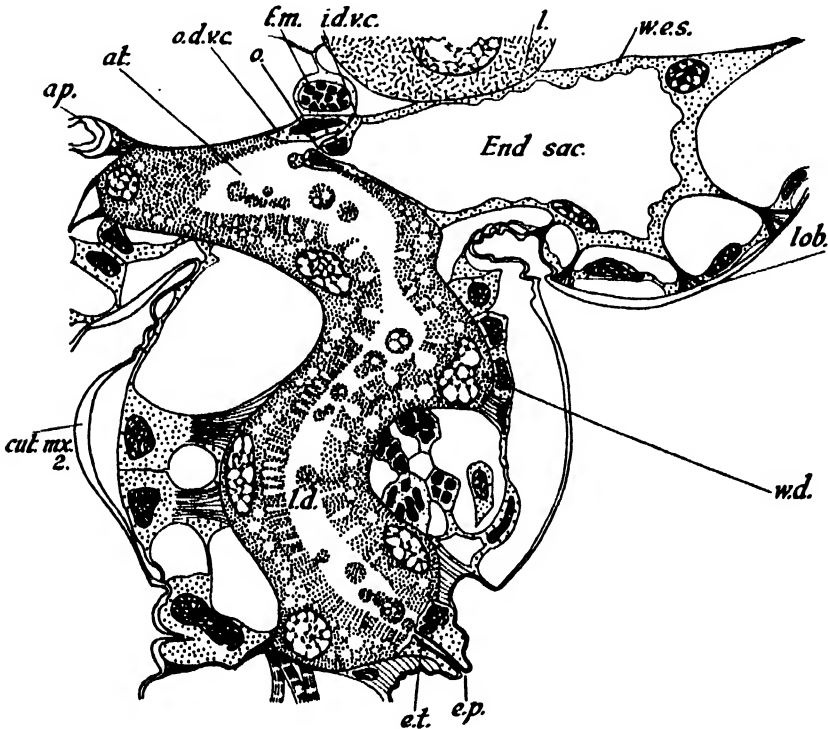


FIG. 1.—Composite diagram from series of parasagittal sections through larval maxillary gland. ($\times 400$ approx.) ap., apodeme forming part of endoskeletal plate; at., atrium of duct; cut. mx. 2, cuticle of basal segment of second maxilla; e.p., excretory pore; e.t., exit-tube; f.m., flexor muscle; i.d.v.c., inner dorsal valve cell; l., liver; l.d., lumen of duct; lob., lobe of body containing end sac; o., opening of valve; o.d.v.c., outer dorsal valve cell; w.e.s., wall of end sac; w.d., wall of duct.

margins of the lobe (lob.) in which it lies; a third coarse strand passes from it dorso-medially and appears to join the sheath of the ventral longitudinal muscle. The "stalk" of the end sac passes medially, and then curves anteriorly and somewhat ventrally to fuse with the dorso-lateral wall of the duct.

The cavity of the end sac communicates with the lumen of the duct (l.d.) by a fine channel (fig. 1, o) shaped somewhat like an hour-glass,

slightly bent at its "waist." This connecting tubule runs approximately parallel to the axis of the animal, and is surrounded by two cells, the inner dorsal (i.d.v.c.) and ventral (v.v.c.) valve cells. Associated with these two cells are the outer dorsal (o.d.v.c.) and ventro-medial (v.m.v.c.) valve cells respectively, the two pairs constituting a tetrahedral sphincter valve, which can be best understood by reference to fig. 2. Prolongations of the lateral regions of the valve cells converge towards a tendinous strand of connective tissue (l.t.c.), which passes along the wall of the end

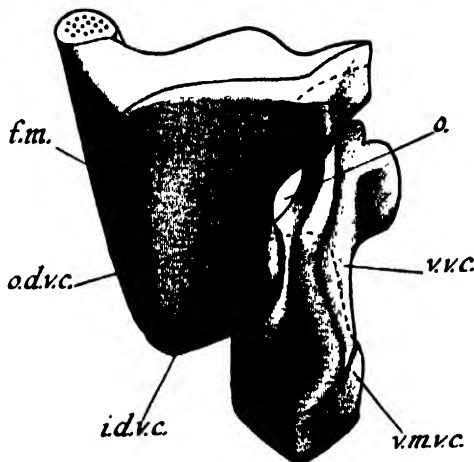


FIG. 2.—Reconstruction of Right Sphincter Valve from postero-lateral aspect. ($\times 1650$ approx.) f.m., flexor muscle; i.d.v.c., inner dorsal valve cell; o., opening of valve; o.d.v.c., outer dorsal valve cell; v.m.v.c., ventro-medial valve cell; v.v.c., ventral valve cell.

sac for a short part of its length and is attached to the neighbouring ectoderm, at the angle between the base of the second maxilla and the lateral body wall (fig. 3). The medial margins of the valve cells approximate within the dorsal wall of the duct and there connect with a tendinous strand (m.t.c.) which extends along this wall medially to join the endoskeletal plate (ap.) and ectodermal tendon bar (e.t.b.) in the neighbourhood of the nerve cord (fig. 3, n.c.). The last-mentioned tendinous strand is prolonged dorso-laterally into a flexor muscle (f.m.) which is inserted into the dorsal face of the outer dorsal valve cell, and thence passes in a dorso-lateral direction to join the ectoderm in the region of reflexion of the carapace fold (fig. 3, car.).

Each valve cell consists of a thin layer of vacuolated hyaline cytoplasm enveloping a large deeply staining nucleus, which resembles markedly the nuclei of the endoskeleton and ectodermal tendon-bar.

Within the inner dorsal and ventral valve cells, between the lateral

and medial tendinous strands, extend respectively vertical and horizontal bundles of myofibrils, which, together with those of the flexor muscle, constitute a triangle around the posterior end of the connecting tubule. In some sections there appeared to be fibrils within the dorsal and ventro-medial cells, but their presence could not be clearly established; if present, they would form the two sides of a second triangle around the

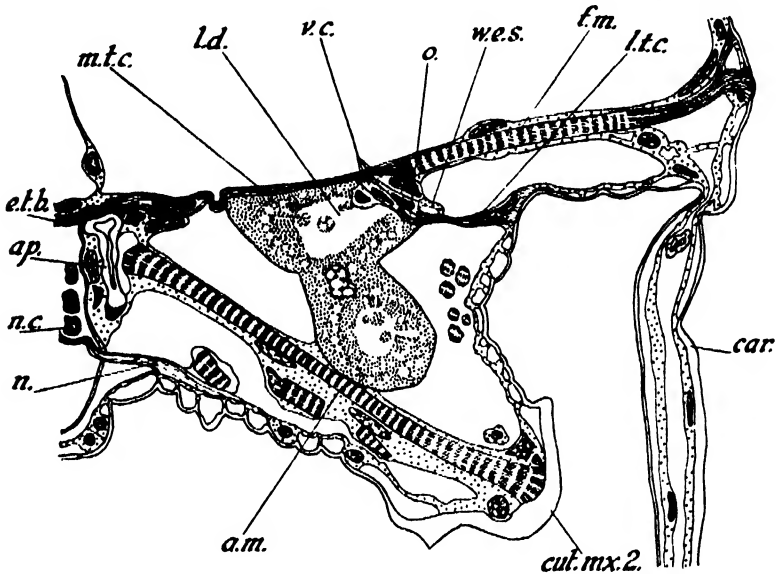


FIG. 3.—Composite diagram from series of transverse sections through larval maxillary gland. ($\times 300$ approx.) a.m., adductor muscle; a.p., apodeme forming part of endoskeletal plate; car., carapace; cut. mx. 2, cuticle of base of second maxilla; e.t.b., ectodermal tendon bar; f.m., flexor muscle; l.d., lumen of duct; l.t.c., lateral tendinous connection; m.t.c., medial tendinous connection; n., nerve; n.c., nerve cell; o., opening of valve; v.c., valve cells; w.e.s., wall of end sac.

anterior end of the connecting tubule, the flexor muscle again providing the third side.

The duct is relatively short, its swollen first part, or "atrium" (at.), extending between the liver and the base of the second maxilla, beyond the anterior border of which it projects slightly (fig. 1). From the postero-ventral region of the atrium, the duct passes into the basal joint of the second maxilla, curving in an S-bend first postero-laterally, then antero-medially, and finally postero-laterally; the more distal region is again slightly dilated.

The walls of the duct (fig. 1, w.d.) are formed by a syncytium, including ten large irregularly shaped and very lightly staining nuclei. In this syncytium three concentric layers can be distinguished. Peri-

pherally, the cytoplasm is very dense, and in some sections shows radial striations due to the presence of either fibrillæ or fine tubular vacuoles. The intermediate layer is irregularly vacuolated and sharply demarcated from the inner layer, which forms the lining of the duct and, under low power, appears as a striated border. With higher magnification it is seen to consist of innumerable radially arranged vacuoles which in some regions are very fine and tubular. In other regions they are flask-shaped and appear to be concerned with the extrusion of secretory matter, which, in the form of finely granular globules, hangs from the walls or lies free within the lumen of the duct.

Distally the lumen of the duct opens into a short dorso-ventrally compressed exit-tube (e.t.), which traverses a papilla directed postero-ventrally, and arising at the distal end of the joint from a depression in its postero-lateral margin. At the tip of the papilla is a slit-like excretory pore (e.p.). The walls of the exit-tube are formed by two cells containing U-shaped nuclei resembling, in size and staining properties, the nuclei of ectodermal cells and curving around the lateral aspect of the proximal end of the exit-tube. The hyaline cytoplasm is vacuolated, and bounded by a thin cuticular layer (fig. 1).

DISCUSSION.

In a consideration of the segmental excretory organs of Crustacea, the problem concerning the homologies of the various parts is important. The extensive literature which deals with this question has been summarised by Woodland (1913), by Burian and Muth (1921), and recently by Needham (1942).

The foregoing account demonstrates that typical maxillary glands are present in larvæ of *Euphausia superba*. The end sac is undoubtedly the homologue of similar organs which, throughout the Arthropoda, wherever examined embryologically, have been shown to arise from mesoderm.

The embryological derivation of the sphincter valve is more difficult to determine, and in the majority of Crustacea the homology of this structure is still a matter for speculation. Needham, in his paper on the segmental excretory organs of *Asellus* (1942), discusses the question and mentions (p. 229): "The discovery of Cannon (1940) that a distinct exit-tube, and a sphincter derived from the end sac, exist in the paucicellular * antennal organ of *Gigantocypris*." This statement is incorrect,

* A term coined by Needham (1942) to indicate "a duct of a few (3 or 4) large cells with intracellular lumen . . ." (p. 206).

for Cannon nowhere makes reference to the origin of the valve cells in *Gigantocypris*. Later in the same paper, Needham says "most workers trace it" (the sphincter) "to the end sac." The statements of some observers that the entire gland is mesodermal in some species (Cannon, 1926; Cannon and Manton, 1927; Hickman, 1936) may be understood to imply that the valve, as part of the gland, is also mesodermal. Other workers (Vejdovský, 1901; Waite, 1899) who saw the valve cells forming part of the wall of the end sac have openly or tacitly assumed that they develop from the coelomic rudiment. A scrutiny of the literature reveals that Needham is the only worker who (in one species) has traced the actual development of the valve cells from the end-sac rudiment, but, unfortunately, owing to the complexity and, at the same time, the semi-diagrammatic nature of his figures, his demonstration is not entirely convincing. Thus it appears that there is no conclusive evidence for Needham's assumption (p. 223) that the valve cells "of most other Crustacean segmental organs, at any rate those of the multicellular type," arise from the end sac. Manton states that the valve cells of *Hemimysis* are derived from the mesoderm of the duct rudiment—her figures (1928, p. 428; text-figs. 28, *a* and *b*), however, impress the reader with the resemblance of the nuclei of the valve to those of the ectoderm. Vogt (1935) demonstrated, in the multicellular antennal gland of Mysids, the origin of a sphincter valve from a mesodermal rudiment distinct from those of both the end sac and the duct. His diagrams (p. 496, text-fig. 18), however, present a significant resemblance to those of Cannon (1925, p. 10, text-fig. 4) showing the *ectodermal* origin of the valve in the paucicellular maxillary gland of Cyprids.

The sphincter valve of *Euphausia superba* strikingly resembles the last-mentioned Cyprid valve, although the former consists of four and the latter of three cells. Professor Graham Cannon, in conversation, has pointed out that it is possible that he overlooked a fourth cell. Both valves are tetrahedral in shape and have similar connections with ectodermal structures. Moreover, the dorsal valve cell is intimately associated with a flexor muscle, the position and connections of which suggest that it is an ectodermal structure originating in much the same manner as the ectodermal flexor muscles of typical thoracic segments of *Hemimysis* (Manton, 1928). It therefore seems probable that the sphincter valve of *Euphausia superba* and the associated flexor muscle originate from ectodermal tissues in connection with endoskeletal structures.

Special interest attaches to the duct of the larval maxillary gland of *Euphausia superba*. Its wall—a syncytium containing ten nuclei—

presents a type intermediate between those conveniently named paucicellular and multicellular by Needham. The two categories were first introduced by Burian and Muth (1921) and elaborated by Cannon who suggested (1924) and subsequently proved (1925, 1926) that in some cases at least the two types of duct are not completely homologous, the paucicellular, intracellular type being ectodermal with an ectodermal valve (Cannon and Manton, 1927, p. 446), the multicellular type being mesodermal. Thus, if the valve of the gland under consideration is indeed ectodermal, the duct would have to be regarded as paucicellular and intracellular, and the larger number of cells might be explained by the fact that these larvæ are relatively large animals. Needham, however, insists on the fundamental homology between the two types of duct and possibly also between the ducts and the nephridia of other groups of animals, postulating "a movement of the presumptive nephridioderm, during the evolution of the Crustacea from the ectoderm to the mesoderm" (p. 239). The intermediate character of the duct of the antennal gland of *E. superba* might furnish support for this hypothesis. The latter does, however, seem to be based on certain misconceptions; for on pages 228-9 Needham writes: "It has never been explicitly stated that the intracellular lumen of paucicellular ducts develops from vacuoles within the cell, so that it is possible that it arises as a groove in the side of each cell and is therefore not truly intracellular." Apart from the fact that evidence is lacking in support of the suggestion that a groove ever does arise along the side of a single cell, a process which could only occur with great difficulty, the first statement in the quotation is incorrect. Cannon, describing the developing duct of a Cyprid (1925, p. 15), has stated that "there were indications in the cytoplasm of this ingrowth, of minute channels that undoubtedly represented the beginnings of the lumen of the duct system." Warren (1938, p. 268), discussing the development of the duct in the antennal gland of *Artemia*, has also noted its intracellular origin; but the work of this author is dubious.

About histological structure, Needham states (p. 209) that there is "a characteristic brush border (Newell and Baxter, 1936) on that surface of the duct cells facing the lumen." This, I believe, is the first time that the term "brush border" has been applied to this layer, which has been variously called a cuticle (Grobbs, 1880), an alveolar or vacuolated layer (Marchal, 1892, p. 109; Vejvodský, 1901; Waite, 1899, p. 165), and a "Stäbchensaum" (Bergold, 1910). If the term is defined in the sense in which it was used by Metalnikoff (1896, after Ehrenburg in Winterstein) as a free border in which filaments merge into flask-shaped vacuoles, then it fits my observations on the maxillary gland of *Euphausia superba* and

the antennal gland of *Marinogammarus marinus* (1941, text-fig. 2) and those of Marchal, Vejvodský, and Waite. On the other hand, Newell and Baxter (1936) mean by "brush border" a free cell border consisting of simple filaments, and this definition does not apply to the above-mentioned observations.

SUMMARY.

1. The larvæ of *Euphausia superba* possess a pair of typical maxillary segmental excretory organs, each consisting of end sac, sphincter valve, duct, and exit tube.

2. The histological characters of the gland as it appears in the fourth furcilia are described.

3. The sphincter valve is a tetrahedron composed of four cells whose appearance and connections suggest an ectodermal origin. Within some of the cells are myofibrils.

4. The duct, a syncytium containing ten nuclei, is an intermediate type between the paucicellular and multicellular categories.

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XXVIII.—Hybrid Sterility in Artificially Produced Recombinants between *Drosophila melanogaster* and *D. simulans*. By **G. Pontecorvo,*** Institute of Animal Genetics, University of Edinburgh, and Department of Zoology, University of Glasgow. *Communicated by* Professor E. HINDLE, F.R.S. (With One Table and Three Text-figures.)

(MS. received October 26, 1942. Read December 7, 1942.)

Drosophila melanogaster and *D. simulans* constitute one of those cases in which two almost identical phenotypes result from different genotypes. In particular, the histology of gametogenesis shows no detectable difference between the two species, nevertheless, the development of the gonads in their hybrids is so upset that invariably sterility results. In these hybrids the germ cells never go beyond the stage of oogonia or spermatogonia.

A special technique (Muller and Pontecorvo, 1940; Pontecorvo, 1942) makes it possible, however, to overcome this sterility and obtain the same kinds of "recombinants" as those which would arise from a backcross of the F_1 hybrids to the *melanogaster* parent, should this backcross be possible. We shall call these recombinants "partial hybrids" to distinguish them from the F_1 "full hybrids" carrying, if diploid, one, or if triploid, two, whole chromosome sets from *melanogaster*, and one whole chromosome set from *simulans*.

Out of the twelve diploid recombinations between the three major chromosomes of the two species possible with the technique adopted, only eight are viable enough to reach the imago stage (Pontecorvo, 1942). Thirty-six imagines of these eight types have been obtained and 25 were investigated (Table I). On the other hand, of the possible types of diploid and triploid full hybrids, it is well known (Sturtevant, 1929) that only those carrying a *simulans* X chromosome reach the imago stage. Several hundred imagines of these full hybrids, including triploids and intersexes, have been obtained.

The present paper deals with the comparative histology of the gonads in the two species, in the eight types of viable partial hybrids, and in four types of viable full hybrids (diploid males and females, intersexes and

* Work done while holding a grant from the Rockefeller Foundation, New York.

triploid females). Dr Koller made some preliminary observations on partial hybrids obtained by Muller and the writer in 1940. The additional material collected since then has made possible the present fuller investigation.

HISTOLOGY OF THE GONADS.

The gonads were examined in imagines a few days old either as whole mounts stained with borax carmine, or as sections stained with crystal violet or acetic lacmoid. For the two species, the full hybrids, and certain other species of insects referred to later on, considerable use has also been made of crushed preparations of larval, pupal, and imaginal gonads fixed in acetic alcohol and stained in acetic lacmoid, acetic orcein, or Feulgen, by the techniques of Darlington and La Cour (1942).

Table I shows the stages of gametogenesis reached by each type of imago. In most types gametogenesis does not go beyond the spermatogonia or oogonia stage, as was first observed by Kerkis (1933) for diploid full hybrids. In the type in which the X and III chromosome pairs are of pure *melanogaster* constitution, both sexes reach the stage of fully grown auxocytes (spermatocytes or oocytes, and nurse cells). Finally, in the type in which the X, II, and III chromosomes are of pure *melanogaster* constitution (the Y and one chromosome IV being the only chromosomes from *simulans*), both sexes reach the stage of mature gametes. The male of the latter type, however, has immobile spermatozoa. By breeding the female, which has attached Xs from *melanogaster* and Y from *simulans*, it had been shown by Muller and Pontecorvo (1940, 1941) that in an otherwise *melanogaster* genotype, and cytoplasm, the *simulans* Y cannot substitute the *melanogaster* Y, as far as sperm motility is concerned (*cf.* Neuhaus, 1939). On the other hand, they showed that the *simulans* IV has no appreciable effect on the fertility of either sex when heterozygous, but affects the last stages of spermateleosis in the male when homozygous.

Disregarding these minor effects of the Y and IV chromosomes, it may be seen from Table I that there are only three types of development of the gonads: (a) *gametogenesis carried to completion*, in either pure species, and in partial hybrids with X, II, and III chromosomes of pure *melanogaster* constitution; (b) *gametogenesis arrested after the production of auxocytes*, in partial hybrids with X and III chromosomes of pure *melanogaster* constitution; and (c) *gametogenesis arrested with the production of oogonia or spermatogonia*, in all other cases.

Oogenesis.—In females with oogenesis carried to completion the newly hatched imago shows ovarioles with many immature follicles

(figs. 1, A and 2, A). The germarium, or terminal chamber, of each ovariole contains very few large primary oogonia in its distal end, *i.e.* close to the filament. These give origin by division to smaller oogonia that go on dividing with strict synchronization. When sixteen products are available the small and flat cells of the pedicel, of mesodermal origin, surround them with a single layer. In the follicle thus formed, the proximal cell (caudad) becomes the oocyte and the other fifteen the nurse cells. By repetition of this process an egg string is formed in which the follicles are more and more advanced in development proceeding from the terminal chamber towards the calix. The enormous growth of the nurse cells and, later, of the oocyte is finely adjusted with the multiplication of the follicle cells which continue to form a single layer. The zygotene stage of meiosis is not observable before the oocyte has attained a size between one-quarter and one-half of that of the mature egg.

In the female with X and III chromosomes of pure *melanogaster* constitution (figs. 1, B and 2, B), on the other hand, there is no trace of synchronous division of the oogonia preliminary to the formation of the follicle. Some isolated oogonia start their transformation into nurse cells—with typical features of both growth and endomitosis—within the terminal chamber; other oogonia in variable numbers are enclosed into follicles which ultimately contain from one to many nurse cells, obviously at different degrees of development, and from none to many oocytes. The follicles are surrounded by irregular layers of follicle cells. Degeneration begins too early for meiosis to take place.

Finally, in the females of all other viable types of partial and full hybrids, including triploids and female-like intersexes, no synchronized division of the oogonia is apparent and no transformation of the oogonia into oocytes and nurse cells ever takes place: the oogonia go on multiplying and fill the terminal chamber with a tightly packed mass of cells (figs. 1, C and 2, C). The description given by Kerkis (1933) for the ovaries of full hybrid diploid females is applicable to all hybrids in this group (see also Schultz and Dobzhansky, 1933).

Spermatogenesis.—Let us now examine the corresponding series of males. The testis of the imago (fig. 3, A), in which spermatogenesis is carried to the stage of production of mature spermatozoa, is divided into three zones: the spermatogonia are limited to the very tip; then follows a region of first spermatocytes; and finally there is a region of later stages of spermatogenesis usually occupying more than half the testis. Stages of the second division are almost exclusively found near the testicular wall on the inner side of the concavity of the spiralized testis. As in the

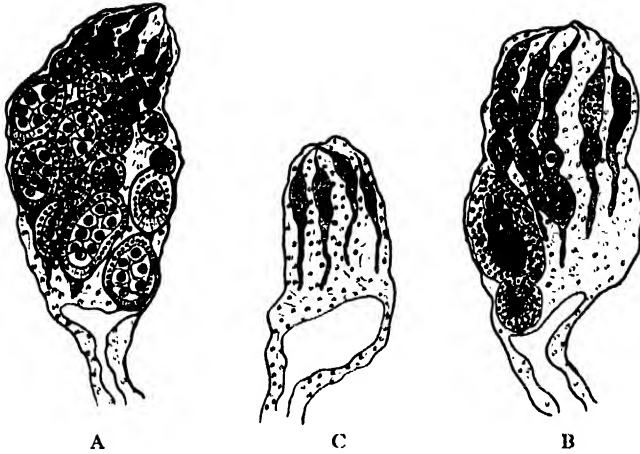


FIG. 1.—Sagittal sections of ovaries, from camera-lucida drawings, all to the same scale. A, ovary of newly hatched females *D. melanogaster*, *D. simulans*, or partial hybrid with X, II, and III chromosome pairs of pure *melanogaster* origin. B, ovary of female with X and III chromosome pairs of pure *melanogaster* origin. C, ovary of any other type of full and partial hybrids, including triploid females and female-like intersexes.

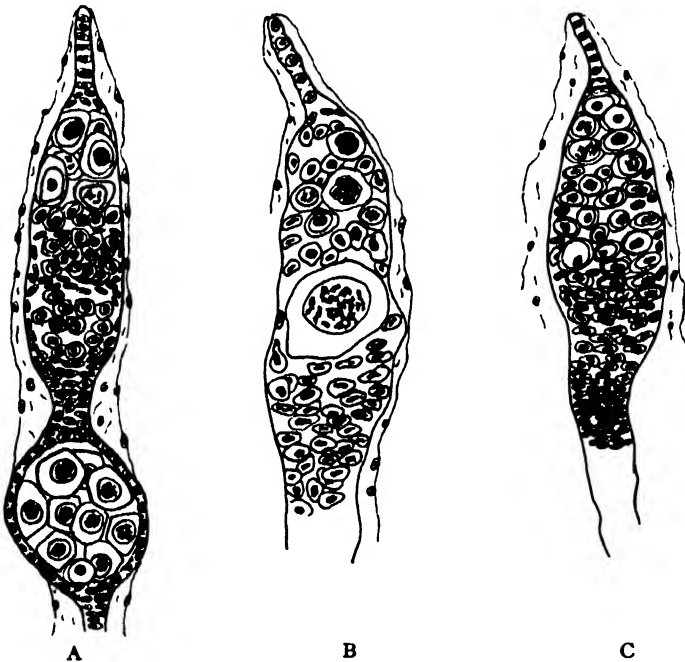


FIG. 2.—Sagittal sections of distal end of ovarioles. A, B, and C as in fig. 1.

female, there is a strict synchronization of the premeiotic (spermatogonial) divisions as well as of the meiotic divisions and of all the processes of spermateleosis. Meiosis takes place in groups of 16 spermatocytes so that 64 spermatozoa are present in each bundle.

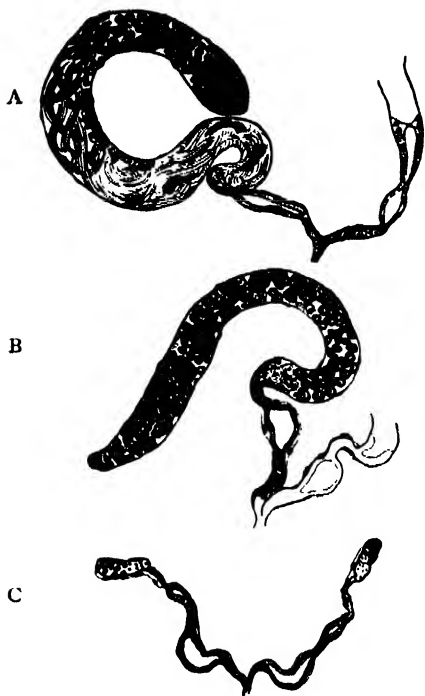


FIG. 3.—Sagittal sections of testes from camera-lucida drawings, all to the same scale. A, testis of males *D. melanogaster*, *D. simulans*, or partial hybrids with X, II, and III chromosomes of pure *melanogaster* origin. B, testis of partial hybrids with X and III chromosomes of *melanogaster* origin. C, testis of all other types of full and partial hybrids, including male-like intersexes.

In most species of insects the spermatogonial divisions and the meiotic and spermateleotic processes take place with strict synchronization in groups of germ cells. Each group is usually contained in a clearly defined cyst. In *Drosophila*, however, the cysts are not so evident. Accordingly we have investigated the formation of cysts in six other species of higher Diptera (*Psila rosæ*; *Psila fimetaria*; *Lucilia sericata*; *Syrphus vitripennis*; *Syrphus luniger*; *Helomyza zetterstedtii*). During other work we also examined the Anopluran *Pediculus corporis* in this respect. The process of cyst formation has been found to be remarkably uniform in all these species, including *P. corporis* where, as described by Hindle and Pontecorvo (1942), meiosis is pushed back in the development of the germ track, and is followed by haploid mitoses. In the earliest stage observable a group is formed consisting of two spermatogonia, possibly sister cells, and one interstitial mesodermal cell which tends to surround

the former with cytoplasmic outgrowths. The two spermatogonia divide synchronously a number of times, constant for each species; the mesodermal cell, whose nucleus suggests a high metabolic activity, forms a kind of sheath around the increasing numbers of spermatogonia. This sheath persists throughout all later stages almost up to the emission of the spermatozoa from the testis.

In *Drosophila* no trace can be seen of the morula-like cyst characteristic of the species indicated above and, presumably, of most other insects.

The synchronized spermatogonia form rather flat irregularly stratified groups in which the relationship with the mesodermal elements is not clear. Guyenot and Naville (1929) showed that each group of spermatids is somehow "implanted" upon a single huge cell ("cellule nourissante"). We have been able to observe this relationship already established at the stage of early 1st spermatocytes. It is probably only a matter of difficult observation that its origin cannot be traced further back. In conclusion it seems very likely that the process of cyst formation follows in *Drosophila* essentially the same lines as in other species.

Passing to the partial hybrid male with X and III chromosomes of pure *melanogaster* constitution (fig. 3, B), the testis contains spermatogonia and great numbers of 1st spermatocytes in various stages of growth. No more advanced stage of spermatogenesis can be found and the spermatocytes degenerate. No sudden change in the staining properties of the chromosomes takes place comparable to that which occurs at diakinesis in the spermatocytes of the two pure species: probably the nucleic acid cycle is very abnormal. However, in the rare cases in which the chromosomes show a faint staining one gets the impression that they are dividing without the nuclear membrane breaking down. When a group of spermatocytes can be seen clearly to form a unit, their number is always variable and also their degree of development.

Finally, in all other types of full and partial hybrids, including male-like intersexes, the testis contains only spermatogonia and mesodermal elements. The description given by Kerkis (1933) for full hybrid males applies equally to all these types.

Before proceeding it is important to point out that, even though all imagines, of those classes where several were obtained, had gonads conforming on the whole to the description reported above, the individual degree of disturbance was extremely variable. For instance, in all the males with X and III chromosomes of pure *melanogaster* constitution spermatogenesis reached the stage of 1st spermatocytes, but while in some the testis was as large as in males of the pure species and full of hundreds of spermatocytes, in others it was hardly larger than in full hybrids. In the most extreme case only two spermatocytes were present in one testis and none in the other.

PROCESSES AFFECTED BY THE HYBRID CONSTITUTION.

From the foregoing it is clear that similar, probably homologous, processes are affected in much the same way in both sexes of partial and full hybrids having comparable chromosome constitutions.

We are faced by at least two such processes: one is the synchronization in the premeiotic divisions; the other is the transformation of spermatogonia into spermatocytes, or of oogonia into oocytes and nurse cells.

That these two processes must be to a certain extent independent is shown by the partial hybrids of both sexes with X and III chromosomes of pure *melanogaster* constitution: even though there is no synchronization in the spermatogonial or oogonial divisions the transformation into auxocytes actually takes place. What fails in this type of partial hybrid are the further steps in gametogenesis, first of all meiosis. It seems probable, however, that these failures are at least in part secondary effects due to the lack of synchronization earlier. Through it, follicles, in the female, and cysts, in the male, are formed with irregular numbers of auxocytes, not all at the same stage of development. The fine adjustment between auxocytes and mesodermal elements is thus profoundly disrupted.

Confining our analysis to the two processes of synchronization and formation of auxocytes, we see that with certain chromosomal constitutions only the former is affected, with others both are affected, and finally with certain constitutions neither is affected.

In particular (Table I), the presence of a *simulans* X chromosome affects the process of auxocyte formation: compare *e* with *y*; *e* females or *sc*; *e* males. The presence of a *simulans* III chromosome affects both the process of synchronization and that of auxocyte formation; compare *y*; *bw* with *y*; *bw*; *e* females or *sc*; *bw*; *e* males. The presence of a *simulans* II chromosome affects only the process of synchronization: compare *y*; *e* females and *sc*; *e* males with *y*; *bw*; *e* females and *sc*; *bw*; *e* males. Finally, the presence of a *simulans* IV chromosome or/and Y chromosome affects neither process: see *y*; *bw*; *e* females and *sc*; *bw*; *e* males. The other combinations confirm these relationships.

DISCUSSION AND CONCLUSIONS.

The effects described in the preceding section arise when a particular *simulans* chromosome in single dose, hence capable of expression in heterozygous condition, is present with other *melanogaster* chromosomes and the combination is made in *melanogaster* cytoplasm. The *simulans* chromosome, therefore, interacts either with one or more *melanogaster* chromosomes or with the *melanogaster* cytoplasm. From the present data we have no means of deciding between these two alternatives; reciprocal crosses would be necessary for which suitable stocks are at present unobtainable.

However, interactions causing viability effects and others causing abnormalities of the bristles have been investigated in this same material: they proved to be interactions between the chromosomes of the two species and not between chromosomes of one species and cytoplasm of the other (Muller and Pontecorvo, 1940; Pontecorvo, 1942). We may provisionally assume that the same holds at least for part of the sterility interactions reported above. On this assumption we may attempt a discussion of the possible mechanism at work.

In the first place, it has been shown that different chromosome combinations affect different developmental processes, the effects being far from additive. In the second place, the manifestation of these effects is extremely variable between individuals with the same chromosome combination. There is, therefore, a type of variation which is discontinuous *between* chromosome combinations but continuous *within* each combination.

It may be recalled that (Pontecorvo, 1942), as a result of the analysis of the salivary gland chromosomes of the two species (Patau, 1935; Horton, 1939; Slizynski, 1941), it is improbable that structural changes play any major part in the interactions arising in the hybrids. Excluding structural changes, a mechanism which fits with the preceding facts is that of interactions between "polygenic combinations" located in non-homologous chromosomes of the two species.

Polygenic combinations (Mather, 1941, 1942; Wigan, 1941; Mather and Wigan, 1942), because of their "relational" balance within each population, result in a remarkable heterogeneity in genotypes being made compatible with the uniformity in phenotypes which characterizes populations in nature. This heterogeneity in genotypes, however, would be revealed in hybrids, the polygenic combinations of one species being, of course, not balanced against those of another species. The variability of the manifestation of the disturbances in our hybrids would, therefore, be the consequence of heterogeneity for polygenic combinations of the two parental species.

This possibility finds support in several hitherto unexplained or ignored cases of F_1 hybrids—of material ranging from *Drosophila* to human races—that show a high variability in quantitative characters, even when the average of the character in the two parental populations is the same. This point is now being tested by the use of inbred stocks.

In *Drosophila melanogaster* and *D. simulans* most characters, and in particular the histology of the gonads, are outwardly identical. However, the genotypes responsible for this identity, when brought together in the hybrids, result in changed end products. When the

development of the gonads is analysed it is possible to distinguish more elementary processes, each affected by interactions between pairs or, at most, triplets of chromosomes. This is even more strikingly the case for other processes which are disturbed in the hybrids. Absence of bristles, for instance, results from an interaction between only two chromosomes, *vis.* X of *simulans* and II of *melanogaster*, both acting as incomplete dominants (Pontecorvo, unpublished). Furthermore, effects on viability result from four independent interactions, with different dominance relationships. Three of these arise from pairs or triplets of chromosomes (Pontecorvo, 1942).

Since in these two species visible structural changes cannot play a major rôle, complementary genes, or sets of genes, must be involved. Muller (1939, 1942) has evolved the concept of "transfers of function" precisely for those cases of evolutionary divergence in which the outward appearance of a character is kept unchanged in two diverging groups whilst its genetic background is changed by means of complementary genes. In his suggestive paper, Muller (1942) gives examples of how some such process can be brought about. His conception becomes more easily acceptable, however, and indeed the occurrence of "transfers of function" appears as an inevitable consequence of isolation, should the transfer be between sets of "polygenes" rather than between a few independent loci.

Let us suppose that any one of the developmental processes dealt with is regulated by two sets of polygenes* carried by non-homologous chromosomes, the normal course resulting from a certain adaptive equilibrium between the functions of the two sets, and each of the two sets being, of course, represented by many "balanced" combinations of *plus* and *minus* allelomorphs. The same adaptive equilibrium may be attained by a change, consequent on recombination, in one set *compensated* by a change in opposite direction in the other set.

Transfers of function as drastic as those shown by our material can hardly arise in one step: indeed, the coincidence within a population of two extreme but still compensatory combinations must be extremely rare. In addition, combinations like those causing sterility, or other drastic incapacitations of the F_1 hybrids, could never have a chance of becoming established.

On the other hand, combinations whose effects are on the borderline of fitness must arise very frequently: indeed their occurrence is one of

* We use the term "set of polygenes" to indicate a complex of genes physiologically and genetically linked, and "polygenic combination" to indicate any one of the combinations of *plus* and *minus* allelomorphs in any one set which may occur in a population.

the features of polygenes. Slightly disadvantageous combinations of this kind are always present and continuously produced in any population. Moreover, the disadvantage of one combination in one polygenic set would be cancelled by frequently available combinations in the other set, opposite in effect and equally disadvantageous by themselves. This mutual support would favour, under suitable population conditions, the establishment of the two changed combinations. A repetition of several such steps, not necessarily all in one direction, would make the differentiation irreversible and lead to conspicuous disturbances when the "new" and the "old" genotypes are again brought together. It is interesting that Mather (1942, p. 332) has obtained evidence, though not yet conclusive, that compensating recombinations may occur. In his case the recombinations took place within one chromosome pair, but this of course is not essential to the argument.

We see, then, that the above hypothesis provides a single mechanism to explain both the origin of the observed interactions and the variability in their degree of expression.

It has been pointed out that the interactions arising from different chromosome combinations are sharply discontinuous in their effects. This is even more so for the other interactions affecting viability, number of bristles, etc., described elsewhere. It suggests three possible alternatives. One is that few—pairs or triplets—polygenic sets located in non-homologous chromosomes control each of the developmental processes investigated, otherwise the sets would be distributed more or less evenly among all chromosomes and the effects of different chromosome combinations would be qualitatively similar and quantitatively cumulative. Another alternative is that, even though many polygenic sets control each process, during the course of divergence of the two species the gradual steps which ultimately lead to each conspicuous transfer of function are more likely to involve only two or three of them rather than greater numbers. A third alternative is that polygenic sets controlling each process are not distributed at random among all chromosomes: a not unlikely possibility, since the evidence is gradually accumulating that the distribution of genes controlling similar developmental processes is not random.

The technique adopted here is only capable of revealing some of the interactions whose members are located in non-homologous chromosomes. It is to be expected, however, that many others would be detected by some method making possible the analysis of intrachromosomal differentiation.

SUMMARY.

1. The histology of the gonads has been investigated in imagines of *Drosophila melanogaster* and *D. simulans*, in their diploid, triploid, and intersex hybrids and in artificially produced recombinants between the two species.

2. Different combinations of chromosomes of the two species have different effects on the course of gametogenesis. The effects are sharply discontinuous between different chromosome combinations and very variable between individuals with the same combination. Homologous processes are affected in much the same way in both sexes.

3. Among the processes which are affected by the hybrid constitution two can be analysed: (a) the synchronization of the premeiotic divisions which leads to groups of 16 coeval oogonia or spermatogonia; and (b) the transformation of the latter into auxocytes. Some chromosome combinations affect only (a), others both (a) and (b), and certain combinations neither.

4. It is suggested that each of the disturbances arises from an interaction between pairs, or in any case between very small numbers, of "polygenic sets" carried in non-homologous chromosomes. In the course of divergence of the two species "transfers of function" must have occurred as the result of compensatory recombinations in pairs of polygenic sets jointly controlling each of the affected processes. The common occurrence of related systematic groups in which the outward appearance of a character is identical in the two groups but disturbed in their hybrids is expected on the basis of such a mechanism.

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XXIX.—The Effects of Radiation on Pollen Grain Development, Differentiation, and Germination. By P. C. Koller, Ph.D., D.Sc., Institute of Animal Genetics, University of Edinburgh. (With Nine Text-figures, One Plate, and Ten Tables.)

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CONTENTS.

	PAGE		PAGE
1. INTRODUCTION	398	ON THE DIFFERENTIATION OF	
2. THE RATE AND TIMING OF		POLLEN GRAINS	415
POLLEN GRAIN MITOSIS	398	6. THE EFFECT OF RADIATION ON	
3. THE EFFECT OF RADIATION		POLLEN GRAIN GERMINATION	420
ON THE RATE AND TIMING		7. DISCUSSION	424
OF POLLEN GRAIN MITOSIS	402	8. SUMMARY	426
4. THE QUALITATIVE EFFECT OF		9. ACKNOWLEDGMENTS	428
RADIATION ON POLLEN GRAIN		10. REFERENCES TO LITERATURE	428
MITOSIS	409	11. DESCRIPTION OF PLATE	429
5. THE EFFECT OF RADIATION			

1. INTRODUCTION.

Tradescantia pollen grains are excellent material for the analysis of X-ray effects. The influence of radiation on cell development, nuclear differentiation, as well as on chromosome structure can be studied. Extensive quantitative analyses, which primarily concern the mechanism of chromosome breaks and reunions induced by radiation, have already been made (Riley, 1936; Sax, 1938, 1940, 1941; Fabergé, 1940). The present paper contains data which show the effects of radiation on cell development, chromosome behaviour, nuclear differentiation, and pollen germination. It is expected that the data obtained will throw some light on the intricate mechanism present within the cell and show that the governing centre of this mechanism is the nucleus.

2. THE RATE AND TIMING OF POLLEN GRAIN MITOSIS.

The time required between telophase of the second meiotic division and metaphase of P.G. (pollen grain) division was found to be 9 days in plants of the same clone grown on the grounds of John Innes Institution, London, in July 1941. Occasionally anthers with dividing P.G.s were

encountered 10 days after meiosis. This period can be shortened considerably if the plants are kept at 24° – 25° C., and prolonged if the temperature is low (Darlington and La Cour, unpublished). Fig. 1 shows the longest and shortest time required by the different phases of P.G. development, when the plants are kept outdoors, and the range of temperature is between 16° – 23° C. The time-table was reconstructed from data obtained by Sax and Edmonds (1933), Mather (1937), Sax (1938, 1939, 1940), Newcombe (1942), and Koller (unpublished).

The difference in development between anthers of the same bud, *i.e.* sister anthers, is expressed (1) in the proportion of non-dividing and dividing cells, and (2) in the proportion of cells before and after P.G. mitosis. Data presented in Table I were obtained from plants C/25 and C/27 and illustrate these differences. Because the plants were kept in identical environments, and the anthers of flower-buds were fixed at the same time, the data obtained are closely comparable. The relatively small proportion of P.G.s with one nucleus, *i.e.* cells before P.G. division, show that P.G. mitosis is well advanced in the anthers of the three buds. While in plant C/25 the developmental differences are expressed mainly in the different proportions of cells before and after P.G. mitosis (compare anthers A and F), in plant C/27 a greater variation was observed in both directions (compare anthers A and D in C/27*a* and A and C in C/27*b*). In many other plants even greater developmental differences were found between sister anthers. Anthers were also encountered in which the P.G. mitosis was almost completed by all the cells, while in the adjacent sister anthers only a very small proportion of the cells had been through division, or *vice versa*.

The rate of P.G. division in an anther can be measured by the increase in the number of P.G.s with two nuclei in a given time. Owing to the developmental differences between sister anthers the rate of P.G. division differs in the different anthers. In order to estimate the rate, the frequency

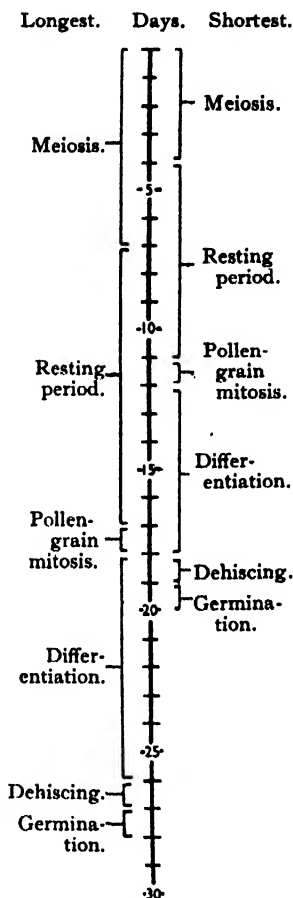


FIG. 1.—Time-table showing the longest and shortest duration of the various stages of P.G. development in *Tradescantia* (at 16° – 23° C.)

TABLE I.—THE FREQUENCY OF THREE STAGES IN DIFFERENT ANTHERS.

Plant.	Antthers.	Total No. of P.G.	P.G.s with one Nucleus.	P.G.s in Mitosis.	P.G.s with two Nuclei.
C/25 (14-7-41)	A	311	11.4	44.2	44.4
	B	243	16.0	55.9	28.0
	C	198	19.2	40.4	40.4
	D	249	20.8	53.8	25.3
	E	348	31.6	39.0	29.5
	F	358	36.8	49.7	13.4
C/27a * (14-7-41)	A	122	9.0	14.8	76.2
	B	116	15.5	45.7	38.8
	C	174	26.5	59.8	13.7
	D	140	27.2	63.5	9.3
C/27b * (14-7-41)	A	196	11.3	37.1	51.6
	B	163	23.4	58.2	18.4
	C	234	38.1	56.4	5.5
	D	237	38.4	45.5	16.1

* Two different buds of the same inflorescence.

of various stages of P.G.s was determined in the two halves of the same anther at 5 hr. interval (Table II).

In the two sister anthers of plant C/23, the increase in the number of P.G.s with two nuclei is 15.3 per cent. and 13.1 per cent. respectively. In one anther of plant C/29 the increase was found to be 23.9 per cent. By comparing these data it was observed that the amount of the increase is related to the number of P.G.s before mitosis found 5 hr. earlier in the anther. It can be seen that in plant C/23 about 25 per cent. of the cells are still before mitosis; the rate of increase after 5 hr. is 13.1 per cent.; in plant C/29, 49.5 per cent. of the cells have not yet completed the division; the rate of increase is 23.9 per cent. Similar data were obtained in other plants.

It was furthermore found that while the number of dividing P.G.s is decreased after 5 hr. in C/23, it is increased in plant C/29. It seems that the proportion of non-dividing/dividing (ND/D) P.G.s is also determined by the number of P.G.s before mitosis. In anthers, in which most of the cells have completed division, the ND/D index is high; on the other hand, when only a small proportion of the cells have passed through P.G. mitosis the ND/D index is small.

Our data enables us to reconstruct the complete cycle of P.G. development in the anther. At the beginning of P.G. mitosis only a very small

number of cells divide and the number of binucleate cells is small. This is due to the fact that cells within the same anther differ in development. As time proceeds (which can be shortened or lengthened by changes in temperature) more and more P.G. divide, and the increase in the number of binucleate cells is rapid. When about two-thirds of the cells have undergone P.G. mitosis, the number of dividing cells and the rate of increase in the number of binucleate P.G.s decreases rapidly. A similar cycle takes place in the sister anthers, but, owing to the developmental differences, these cycles differ at any given time.

When an analysis was made of the relative proportion of division stages in sister anthers, the number of cells in prophase was found to be variable. This variation apparently depends on the phase of the cycle within the anther. When an anther is in the middle of the cycle, as is indicated by the greatest mitotic activity, the proportion of dividing cells in prophase is higher than either at the beginning or towards the end of the cycle. This variation in the proportion of prophase suggests that the time required for the different stages of P.G. division may also be altered during the cycle. At present, however, our data are not sufficient for a definite conclusion.

Further analysis has shown that the proportion of P.G.s in metaphase and post-metaphase stages (anaphase-telophase) is dependent (1) on the number of cells before and after P.G. mitosis, and (2) on the number of P.G.s actually dividing. Table II shows that the PM/M index (indicating the proportion of post-metaphase/metaphase stages) is almost the same in the two halves of the same anther after 5 hr., while at the same time there is a great increase in the number of P.G.s which completed division. It was furthermore found that this index is very similar in sister anthers which otherwise exhibit great developmental differences. The "normal level" of PM/M index in plants investigated during July-August 1941 was found to be between 1.20-1.46.

When P.G.s of sister anthers and those of sister buds of the same plants are compared it can be seen that in many plants they exhibit variation in the same direction. If sister anthers show great developmental differences, very similar differences were found in anthers of other flower buds of the same plant. Such uniform behaviour in sister buds is conditioned genetically and environmentally.

It is very probable that the developmental differences in P.G.s of *Tradescantia* affect the radiation effects on chromosome structure, behaviour, and nuclear differentiation. The consequences of heterogeneity within and between anthers at present, however, cannot be estimated because, so far, all the data used for quantitative analysis have

TABLE II.—FREQUENCY OF VARIOUS STAGES IN TWO HALVES (A AND B) OF THE SAME ANTHOR AT 5 HOURS' INTERVAL.

Plant.	Anther.‡	Total No. of P.G.	Per cent. of Cells before P.G.M.	Per cent. of Cells in Phases.			Per cent. of Cells after P.G.M.	PM. M.*	ND. D.†
				Pro-phase.	Meta-phase.	Post-metaphase.			
C/23 ‡ (16-7-42)	A ¹	327	25.1	30.6	5.2	6.1	33.0	1.17	1.39
	B ¹	367	14.2	26.9	5.2	5.4	48.2	1.05	1.65
	A ²	250	22.2	18.0	5.4	6.0	48.3	1.14	2.28
	B ²	278	16.3	13.1	4.2	5.0	61.4	1.16	3.48
C/29 (21-7-41)	A ²	273	49.5	26.3	6.8	7.1	10.3	1.05	1.50
	B ²	310	24.5	23.1	8.3	9.9	34.2	1.20	1.46

* $\frac{PM}{M}$ index expresses the proportion of post-metaphase/metaphase stages in Pollen Grains.

† $\frac{ND}{D}$ index expresses the proportion of non-dividing/dividing Pollen Grains.

‡ Anthers A¹B¹ and A²B² are taken from different buds.

§ A-half of the anther is taken at 10.30 a.m., B-half at 3.30 p.m.

been collected and grouped together from independent experiments in which observations were made on P.G.s of different anthers, bud and plants, and the environmental conditions were left also uncontrolled.

3. THE EFFECT OF RADIATION ON THE RATE AND TIMING OF POLLEN GRAIN MITOSIS.

Alberti and Politzer (1924) made the first attempt to study the quantitative effects of radiation on the cell. They found that the number of dividing cells is reduced 2 hr. after radiation. This observation was corroborated by Strangeways and Hopwood (1926). They estimated that in tissue culture of chicks the number of dividing cells is 7 times less than in untreated cultures 80 minutes after radiation. Marquardt (1938) was able to observe that X-rays not only suppressed division, but if cells in division were exposed to radiation the duration of mitosis is increased. Carlson (1941) found that X-rays affect the various stages of division in a different way and to a different degree.

It is difficult to obtain reliable data from quantitative analysis of X-ray effects on a cell population owing to the developmental differences between cells at the time of radiation. This fact explains why many investigations lead only to ill-defined generalisation. It has been shown previously, that in spite of the developmental differences, the proportion of dividing

P.G.s in metaphase and post-metaphase stages is very similar in sister anthers of *Tradescantia*. Thus PM/M index can be used not only to detect, but also to measure accurately the effect of radiation on the rate and timing of division. In all our experiments only the dividing cells and the relative proportions of division stages are considered when analysing the effects of radiation. Data obtained are given below.

TABLE III.—EFFECT OF RADIATION ON THE TIME OF DIVISION IN POLLEN GRAINS.

Plant.	Dose.	Time in Hours after Treatment.	Per cent. of Dividing Cells in			PM/M	Total Number of Pollen Grains.	Notes.
			Pro-phase.	Meta-phase.	Post-metaphase (Anatelo-phase).			
C/23	Nil	..	72.9	12.5	14.6	1.18	327	(<i>vide</i> Table V.)
C/25	"	..	72.0	11.9	16.1	1.46	801	6 anthers.
C/27	"	..	81.4	8.0	10.6	1.33	264	4 "
C/31	"	..	79.3	9.4	11.3	1.20	266	5 "
90/5	90 r.	2	63.3	16.7	19.9	1.19	251	4 anthers.
90/14	"	4	69.4	14.0	16.6	1.19	307	4 "
90/21	"	7	74.5	12.7	12.8	0.98	365	5 "
90/90	"	96	69.3	15.0	15.7	1.05	293	4 "
360/55	360 r.	1	65.6	19.9	14.8	0.75	138	1 anther } Same flower
360/55	"	2	64.8	20.9	14.3	0.68	282	1 " } bud.
360/48	"	3	43.5	23.8	32.7	1.37	214	5 anthers.
360/50 *	"	5	71.7	18.1	10.1	0.56	237	5 "
360/19	"	5	78.1	11.1	10.8	0.97	279	5 "
360/55	"	5	64.2	18.2	16.9	0.91	124	One anther from same bud as 1 and 2 hours after 360 r.
360/34	"	24	75.0	13.9	11.1	0.80	332	5 anthers.
360/54 *	"	24	72.0	18.4	8.8	0.48	147	5 "
360/36	"	48	68.9	16.2	14.9	0.92	392	5 "

* Anthers of plants 360/50 and 360/54 were fixed on 17-7-41 at 4 p.m.

It was seen that immediately following radiation with 360 r. there is a decrease in the number of P.G.s in prophase. The lowest level was found at 3 hr. after treatment. When only 90 r. was used, the "prophase-suppressing" effect of radiation is very small, if there is any. Another effect of 360 r. is a significant increase in the number of P.G.s at metaphase. The PM/M index 1 and 2 hr. after radiation is 0.75 and 0.68 respectively, which is much below the "normal level" of 1.20-1.46. The change in the relative proportion of PM/M is brought about by an increase in the number of P.G.s in metaphase, and it is not due to a decrease in the number of post-metaphase stages. This fact is shown

by quantitative and qualitative analysis. First of all, the proportions of dividing P.G.s in post-metaphase stages are nearly the same in treated and untreated anthers. Secondly, the qualitative analysis, which will be described below in more detail, has also shown that the most characteristic primary radiation effect in dividing cells is the "clumping" of chromosomes which results in the prolongation of "metaphase" stage.

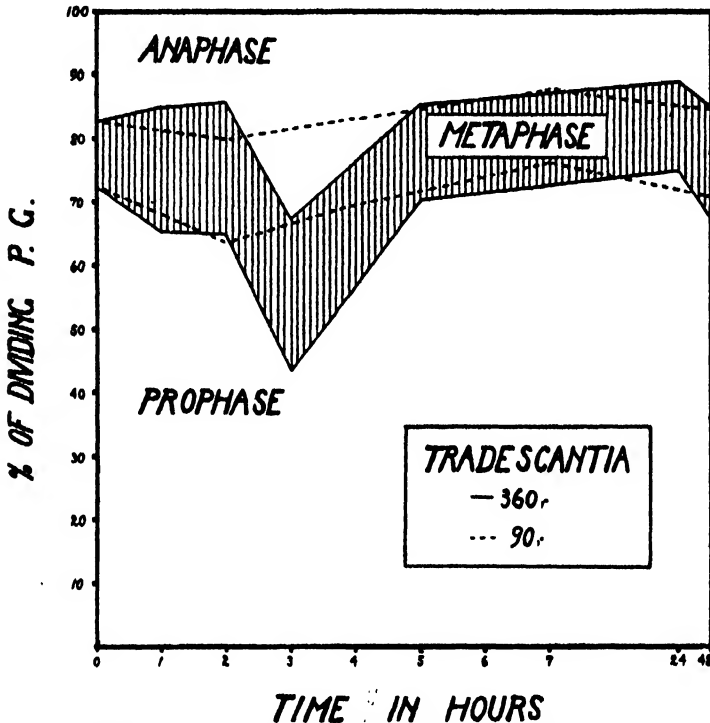


FIG. 2.—Graph showing the "prophase-suppressing" effect of radiation.

This specific effect of radiation with 90 r. is much smaller than with 360 r. It was also observed that with 90 r. the time between treatment and effect is much longer; the "metaphase-prolonging" effect, *i.e.* clumping, comes into operation about 7 hr. after radiation.

The effect of radiation on the various stages of P.G. mitosis is illustrated in figs. 2 and 3. It is shown that 3 hr. after radiation (360 r.), when the "prophase-suppressing" effect is the greatest, the relative proportion of the post-metaphase stages is the highest. This rapid increase is attributed to the fact that those P.G.s in which metaphase is arrested and prolonged 3 hr. after radiation, proceed to anaphase and telophase. This rapid increase in the post-metaphase stages is shown by the PM/M

index, which is the same now as in untreated anthers. The relative proportion of P.G.s at metaphase is, however, still very high, indicating that the "metaphase-prolonging" effect of radiation is continuous. The PM/M index shows that radiation (360 r.) is still effective on metaphase 48 hr. after radiation.

While the effect on metaphase can persist for 48 hr., it was found that the suppression of prophase with 360 r. is restricted to 3 hr. The relative

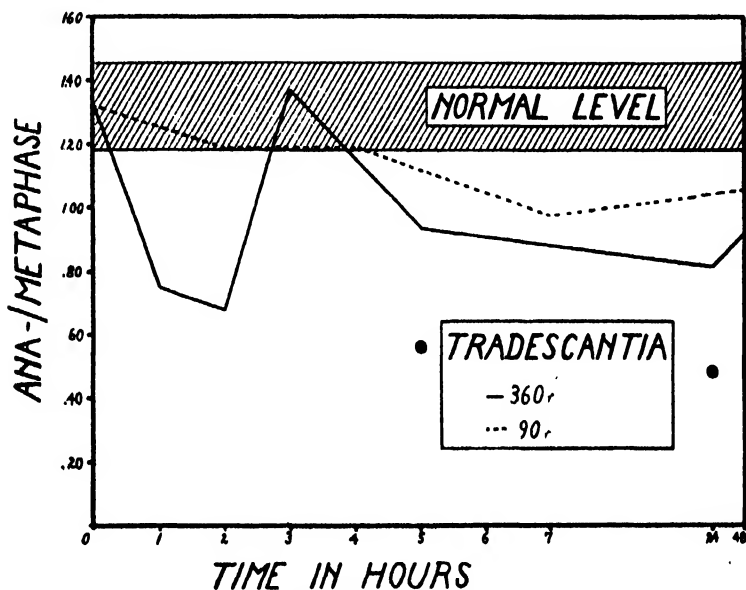


FIG. 3.—Graph showing the effect of radiation on the metaphase of P.G. mitosis. P.G.s of two plants (⊗) fixed at 5 and 24 hr. after radiation have a small PM/M index; it is attributed to difference in temperature at the time of fixation.

proportion of P.G.s in prophase more than 3 hr. after radiation is again the same as it was before radiation. No increase in the number of dividing cells which would indicate a compensation in time follows the suppression of prophase. It is not improbable that the fall in the frequency of prophase immediately after radiation (360 r.) is due not only to the arrest or prolongation of resting stages, but also to the reversion of some prophases to "resting" stage. The fact that diplo-chromosomes, which are the result of reversion (Darlington, 1937), were frequently seen after radiation (White, 1935; Carlson, 1941) indicates that reversion of prophase does not occur after radiation. The absence of diplo-chromosomes prevents us estimating the frequency of reversion if there is any in P.G.s of *Tradescantia*.

During the analysis it was found that the PM/M index in two plants

(360/50 and 360/54, 5 and 24 hr. after 360 r.) was very small, and indicates an unusually long duration of metaphase stage. Anthers of both plants were fixed on the same date and same hour. In P.G.s of other plants treated in the same way but fixed at different times, the PM/M proportion was much higher. The differences in PM/M are due to difference in environment, particularly in temperature at the time of fixation.

The significance of those quantitative differences which were brought about by the radiation on P.G. mitosis in sister anthers was determined

TABLE IV.— χ^2 TEST SHOWING THE EFFECT OF RADIATION ON POLLEN GRAINS IN DIFFERENT STAGES OF DIVISION.

Plant.	Dose.	Hrs. after Treat-ment.	χ^2 Dividing and Non-dividing.	D.f.	P.	χ^2 Resting Stages.	D.f.	P.	χ^2 Dividing Stages.	D.f.	P.	χ^2 of Total.	D.f.
90/5	90 r.	2	21.6400	3	<0.001	35.0262	3	<0.001	2.7638	6	0.90-0.80	59.4390	12
90/14	"	4	3.6270	3	0.30	24.0038	3	<0.001	2.1731	6	0.90	29.8039	12
90/21	"	7	4.0002	3	0.30-0.20	103.6044	3	very small	6.5385	6	0.50-0.30	114.1431	12
90/90	"	96	0.1792	3	0.99-0.98	5.4244	3	0.20-0.10	5.3831	6	0.50	10.9867	12
360/48	360 r.	3	137.3072	4	very small	185.8044	4	very small	13.3663	8	0.10	336.4779	16
360/19	"	5	22.4652	4	0.001	96.4872	4	very small	23.7425	8	very small	142.6949	16
360/34	"	24	12.1435	4	0.02-0.01	21.6030	4	<0.001	7.7853	8	0.50	40.9318	16
360/36	"	48	22.7603	4	0.001	126.6913	4	very small	17.5771	8	0.05-0.02	167.0287	16
C/25	Nil	..	29.9815	5	<0.001	113.0101	5	very small	10.5709	10	0.50-0.30	153.5625	20
C/27	"	..	39.8786	4	<0.001	32.8774	4	<0.001	14.1896	8	0.10-0.05	86.9456	16
C/27	"	..	77.5470	3	<0.001	86.1376	3	<0.001	6.5999	6	0.50	170.2845	12

by calculating the χ^2 and P (1) between proportion of resting-dividing P.G.s, (2) between the three stages of division, and (3) between the proportion of P.G.s before and after P.G. mitosis, using contingency tables. The χ^2 and Ps obtained from the statistical analysis are given in Table IV.

The χ^2 tests show that even when the ND/D index and the relative proportion of mono- and bi-nucleate P.G. are highly variable between sister anthers, the relative proportion of cells in the various stages of division are almost always constant. The effect of radiation on the timing of P.G. mitosis is not dependent on the developmental stages of the anther; it is always expressed in the same way and to the same degree. A similar

behaviour was observed when the radiated anthers of the same bud were fixed at different intervals (Table V).

In the four anthers of plant 360/55, radiated with 360 r., the number of P.G.s before mitosis is nearly the same. Though very similar developmental stages were found in sister anthers of control plants, such a uniform

TABLE V.—THE EFFECT OF RADIATION ON THE RATE AND TIMING OF DIVISION IN POLLEN GRAINS.

Plant.	Dose.	Hours after Treatment.	No. of Pollen Grains.	Per cent. of Cells Non-dividing.		Per cent. of Dividing P.G.s.	Per cent. of Dividing Cells in			ND. D	PM. M
				Before P.G.M.	After P.G.M.		Pro-phase.	Meta-phase.	Post-meta-phase.		
C/23	Nil	0 *	327	43·1	56·9	42·9	72·9	12·5	14·6	1·39	1·18
		5	367	22·8	77·2	37·5	71·7	13·8	14·5	1·65	1·05
360/55	Nil	0 *	144	82·8	17·3	39·6	61·5	17·6	21·6	1·53	1·20
	360 r.	1	138	75·4	24·7	44·3	65·6	19·4	14·8	1·27	0·75
	"	2	282	80·8	19·3	37·3	64·8	20·9	14·3	1·69	0·68
	"	5	124	69·1	30·9	42·8	64·2	18·9	16·9	1·34	0·91

* 0 hour corresponds to 10.30 a.m.

χ^2 TEST OF SIGNIFICANCE.

	χ^2 .		D.f.		P.	
	C/23.	360/55.	C/23.	360/55.	C/23.	360/55.
Dividing-Non-dividing stages .	1·3325	2·3086	1	3	0·30-0·20	0·50
Non-dividing stages . . .	19·9647	5·6370	1	3	very small	0·20-0·10
Between stages . . .	0·2708	3·2007	2	6	0·90-0·80	0·99

development in four sister anthers as was seen in plant 360/55 was never encountered. It is, therefore, reasonable to assume that the decrease or complete obliteration of the developmental differences in sister anthers fixed at different intervals is due to radiation. It was also found that in the sister anthers the proportion of P.G.s before and after division is very similar. These two facts, namely (1) the same proportion of dividing P.G.s and (2) the small increase in the number of binucleate P.G.s in sister anthers, indicate that the whole process of P.G. development may have been slowed down as a result of radiation. However, it is difficult to detect such a universal effect of radiation on all stages

of mitosis, because the "prophase-suppressing" and "metaphase-prolonging" effect of radiation can, and usually do, operate together. X-rays affect various processes of P.G. development, and in the end the results of radiation are a combination of various effects. When these are analysed independently, erroneous conclusions can easily be drawn. It is evident that the "prophase-suppressing" effect of radiation, if it comes into operation immediately, can be detected only 2 hr. after radiation (fig. 4), if we consider only the ND/D index. An increase in the number

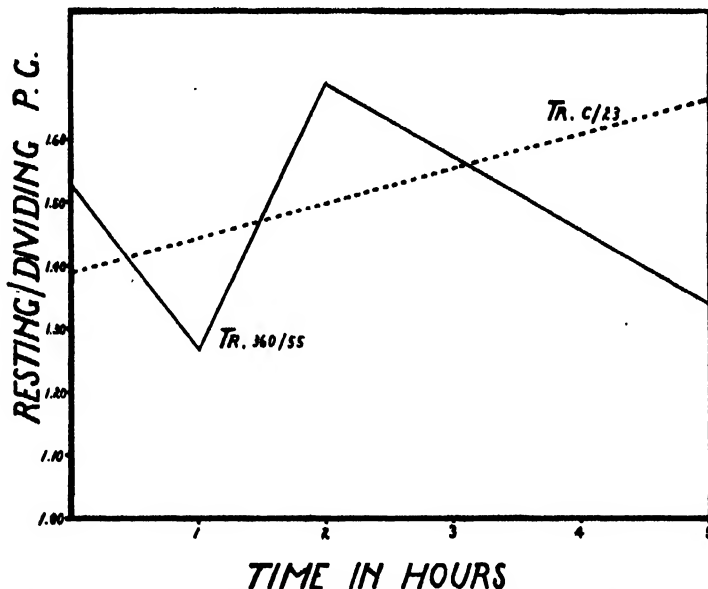


FIG. 4.—Graph showing that the "prophase-suppressing" effect comes into operation 2 hr. after radiation with 360 r. Increase in the number of P.G.s 1 hr. after radiation is due to prolongation of the metaphase stage.

of dividing cells immediately following radiation, however, obscures the manifestation of the "prophase-suppressing" effect. We know now that this increase in the number of dividing cells is brought about by a prolongation of the metaphase stage in cells already in division.

It was pointed out already that when P.G.s are rayed with 90 r. the "prophase-suppressing" effect is very small if there is any (fig. 2). The effect of 90 r. on metaphase can be detected only 7 hr. after radiation (fig. 3). It is expected that an even smaller dose, though it can induce breaks in chromosomes or chromatids, would be insufficient to suppress prophase or to increase the duration of metaphase.

The data obtained on the quantitative radiation effects on P.G. development and division indicate that the end results of these effects are

determined or greatly influenced by the combination of various factors. The developmental differences between sister anthers alone can jeopardise the reliability and comparability of quantitative analyses. Environmental differences, particularly in temperature, exaggerate the developmental differences, hence further diminishes the reliability of the analysis. It is obvious that the developmental differences, prophase suppression, and prolongation of metaphase which follow radiation, should be taken into consideration also when "secondary effects" are quantitatively analysed. Secondary effects observed in P.G.s of sister anthers at a given time after radiation cannot be used alone as the most reliable and only criterion to determine the actual developmental conditions which were present in a cell at the time of radiation.

4. THE QUALITATIVE EFFECT OF RADIATION ON POLLEN GRAIN MITOSIS.

Qualitative effects, such as "clumping" and "stickiness" of chromosomes, were described and classified as "primary effects" of radiation by Alberti and Politzer (1924). Pekarek (1927) found that the expression of primary effects is determined by the dose. Analysis of radiated P.G.s of *Tradescantia* has shown that they are of three kinds. They are expressed by:

- (1) Prolonged congression of chromosomes at metaphase.
- (2) Stickiness of chromosomes and chromatids.
- (3) Disturbed co-ordination between chromosomes and cytoplasm.

The degree of manifestation of each effect is determined by the dose and time after radiation. All the three effects were observed frequently together, which fact suggests either that they may be causally connected, or that they are only different expressions of one change induced by radiation in the structural organisation of chromosomes.

(1) *Prolonged Congression of Chromosomes*.—In P.G.s of untreated *Tradescantia* during metaphase the six chromosomes are placed so that the centromere regions of chromosomes are always turned towards the centre of a well-defined equatorial plate, which is formed between the two poles. Congression of chromosomes, after the disappearance of nuclear membrane, towards the centre of the cell is followed by their orientation in the equatorial plate equidistant from the two poles. It was found that X-rays disturb both processes. Congression of chromosomes is prolonged, chromosomes "clump" together without any orientation, consequently no equatorial plate is formed and the metaphase spindle is absent.

It was seen that 1 hr. after radiation (360 r.) the chromosomes are grouped together in the centre of the P.G. Their lack of orientation is, however, more obvious at that time than the prolonged congression (fig. 5, *a*). Two and three hr. after radiation the chromosomes form a

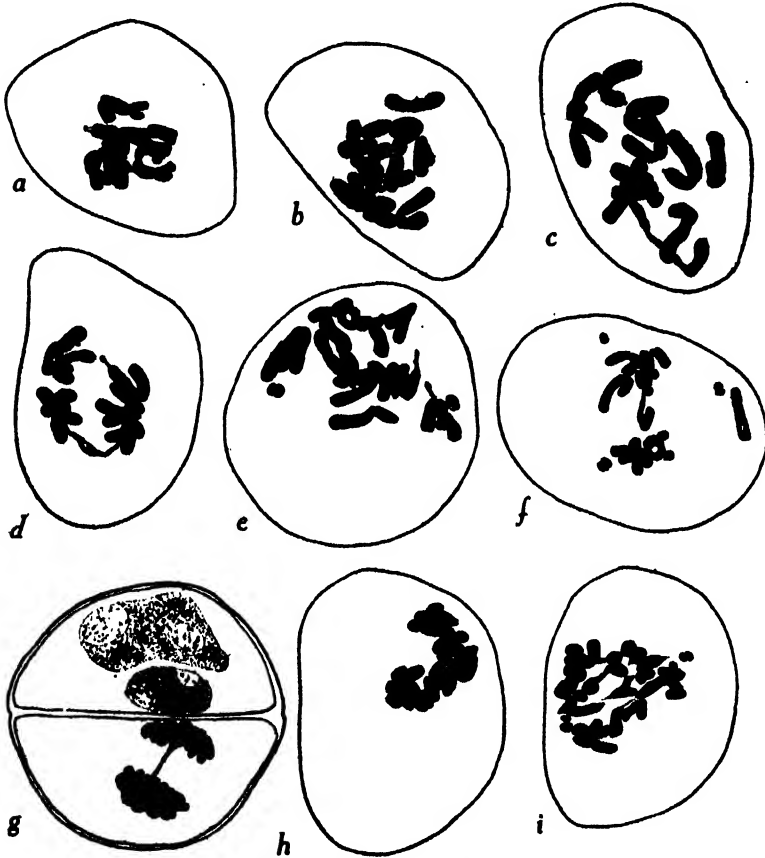


FIG. 5.—The effect of 360 r. on dividing P.G.s. (Description in text. *a*, 1 hr.; *b*, 2 hr.; *c*, *d*, *e*, *g*, *h*, 3 hr.; *i*, 5 hr.; *f*, 7 hr., after radiation.) $\times 1200$.

solid mass, in which no individual members can be identified (fig. 5, *h*). They may form two separate groups (fig. 6, *c*). The abnormal prolongation of congression and stickiness leads to a modified metaphase stage. Metaphase in a cell in which the chromosomes are clumped together may be completely replaced by this stage; the three characteristics of metaphase stage, (1) chromosomes orientation, (2) equatorial plate, and (3) spindle formation, are suppressed. The quantitative repercussions of this effect of radiation on P.G. mitosis and development

has already been discussed; it can now be seen that "prolongation of metaphase stage" is not a direct but an indirect effect of radiation.

The problem is how this prolongation of congression of chromosomes is brought about by radiation. Marquardt (1938) believes that clumping as well as stickiness of chromosomes are due to X-ray induced changes in the colloid condition of the cytoplasm. Shinke (1939) described "chromosome coalescence" induced by external agents such as hypertonic solutions, abnormally high or low temperature, and chloral hydrate, and compared it with clumping following radiation. Shinke attributed this phenomenon to dehydration in the spindle area. Similarly, Sax and Swanson (1941) consider that clumping is a physiological effect of radiation. By analysing prolonged congression of chromosomes in several P.G.s fixed at different times after radiation, it was inferred that it cannot be the result of disturbances in the cytoplasm alone. Because not every chromosome of the P.G. shows the same behaviour after radiation, it was assumed that X-rays must affect processes which are, under normal conditions, co-ordinated not only within, but also between chromosomes.

During the experiments it was observed that 3 hr. after raying with 360 r. the centromere region of some chromosomes in a few P.G.s is attenuated, it is thinner than other regions and stains very slightly with Feulgen's stain (fig. 6, *a*), which indicate that this region is despiralised. In other P.G.s broken chromosomes were seen at meta- and ana-phase. The loci of breaks were at or in the centromere region of the chromosomes (fig. 5, *e*). It was furthermore noticed that chromosomes, which have undergone congression to an extreme degree and stick together at metaphase, are thicker than normal (figs. 5, *h*; 6, *c*, *h*). Their stickiness indicates an overspiralisation. This overspiralisation of chromosomes can be seen even more clearly at anaphase (figs. 5, *b*, *c*, *e*; 7, *a*). Displacement or lack of orientation of whole chromosomes or chromosome arms with centromere was frequently found at metaphase of P.G.s rayed with 360 r. 5 hr. after radiation (fig. 6, *e*, *f*). Displaced chromosomes are less spiralsed. Because centromeres control both internal and external chromosome movements, such behaviour may be attributed to the inactivation of centromere.

(2) *Stickiness of Chromosomes*.—Chromosomes may also become fused and stick together at metaphase. Marquardt (1938) assumed that the "matrix" of chromosomes was affected by radiation, and when the matrices touched they stuck together. Stickiness exhibits great variation. Often chromosomes and sister chromatids stick together by their telomeres only (figs. 5, *c*; 6, *i*). At anaphase the separating daughter chromosomes remain connected by a thin, long thread (fig. 7, *c*, *d*, *e*). At telophase

"false bridges" are common (figs. 5, *g*; 7, *f*, *g*, *h*, *i*). The frequency of P.G.s showing stickiness of chromosomes or chromatids at telomeres only, is very high. Frequently, large segments of daughter chromosomes stick together (fig. 7, *a*, *b*). Stickiness was first seen 2 hr. and disappeared 12 hr. after radiation with 360 r. at 19°–24° C.

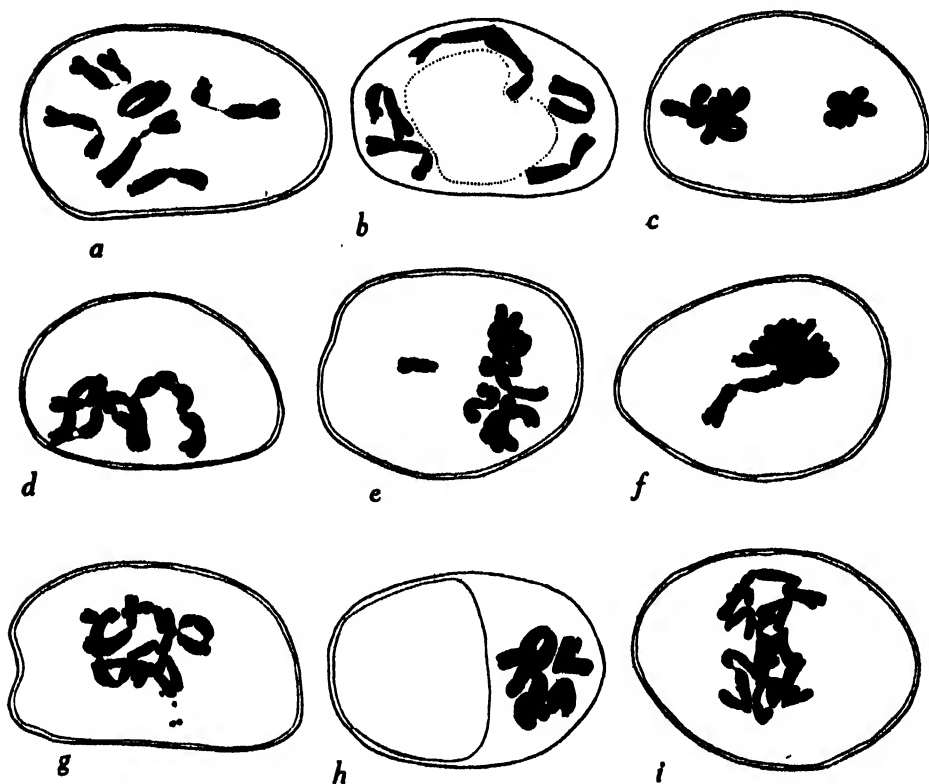


FIG. 6.—The effect of radiation with 360 r. on metaphase and anaphase of P.G. mitosis. (Description in text. *d*, 2 hr.; *a*, *b*, *c*, 3 hr.; *f*, *g*, *h*, 5 hr.; *i*, 7 hr., after radiation.) $\times 1200$.

Stickiness of chromosomes at metaphase was observed without prolonged congression, which suggests that the two phenomena are independent (fig. 6, *d*, *g*). Some chromosomes within the same cell segregate normally, while others stick together and lag at anaphase (fig. 7, *a*, *e*). Similarly, metaphases were found in which chromosomes, though lying side by side, did not stick. This suggests that the physico-chemical organisation is not uniformly affected by radiation in every chromosome within the same cell.

The facts that (*a*) both sticky and non-sticky chromosomes are present

in radiated cells together, (b) stickiness is accompanied by despiralisation of the telomere or telomeric regions (fig. 5, d), (c) stickiness first appears 2 hr. after radiation, suggest that it cannot be due to a change in the cytoplasm only, but is brought about by a change in chromosome organisation itself. It is assumed that X-rays affect the organising centres of chromosomes so that non-polymerised nucleic acid is deposited in excess (Darlington, 1942). The fluid nucleic acid deposit on different chromosomes has a tendency to run together, thus causing stickiness. A similar relationship was found to exist between excessive nucleic acid charge and centromere in the B chromosome of maize (Darlington and Upcott, 1941).

(3) *Disturbed Co-ordination between Chromosomes and Cytoplasm.*—In dividing P.G.s of *Tradescantia* the axis of the meta-anaphase spindle is the shortest diameter of the cell; it lies usually at right angles to the thicker wall (Sax and Edmonds, 1933). The position of the spindle-axis in P.G.s can be altered by radiation. The altered position alone, however, does not interfere with normal segregation of chromosomes. A similar effect on the spindle was induced by temperature (Sax, 1937). Abnormal behaviour of chromosomes at anaphase and irregularities in segregation was found to be always associated either with a complete absence or a delay in spindle formation. In the absence of a spindle after a prolonged metaphase stage, the overspiralised chromosomes fall apart without any orientation (fig. 5, b, c). It is interesting to note that radiation not only can induce prolonged congression, but, on the contrary, it can also completely prevent congression of chromosomes. The "split metaphase" found in a P.G. (fig. 6, b) rayed with 360 r. 3 hr. after treatment is attributed to the suppression of chromosome congression. Frequently, X-rayed P.G.s were encountered at the time of dehiscence with several large "supernumerary nuclei," which stained slightly with Feulgen's stain. Such supernumerary nuclei may have been produced by abnormal chromosome segregation due to the absence of spindle or "split metaphase."

Stickiness interferes with the movement of chromosomes; particularly when large segments are involved it may lead to non-disjunction (fig. 7, a). Chromosomes which stick together are often prevented from normal orientation at metaphase. Abnormal orientation of some chromosomes within the cell is responsible for the abnormal development of the spindle.

It was observed that in the P.G.s of *Tradescantia* at metaphase and anaphase vacuoles develop on each side of the spindle. They disappear after telophase (Sax and Edmonds, 1933). In some P.G.s when rayed with 360 r. the two vacuoles fuse together and form one. The chromosomes in such P.G.s are pushed to one side (figs. 6, h; 7, d). The two

vacuoles may lie separately on the same side of the anaphase spindle (fig. 7, *c*).

Our observations suggest that while X-ray (360 r.) affects all or some

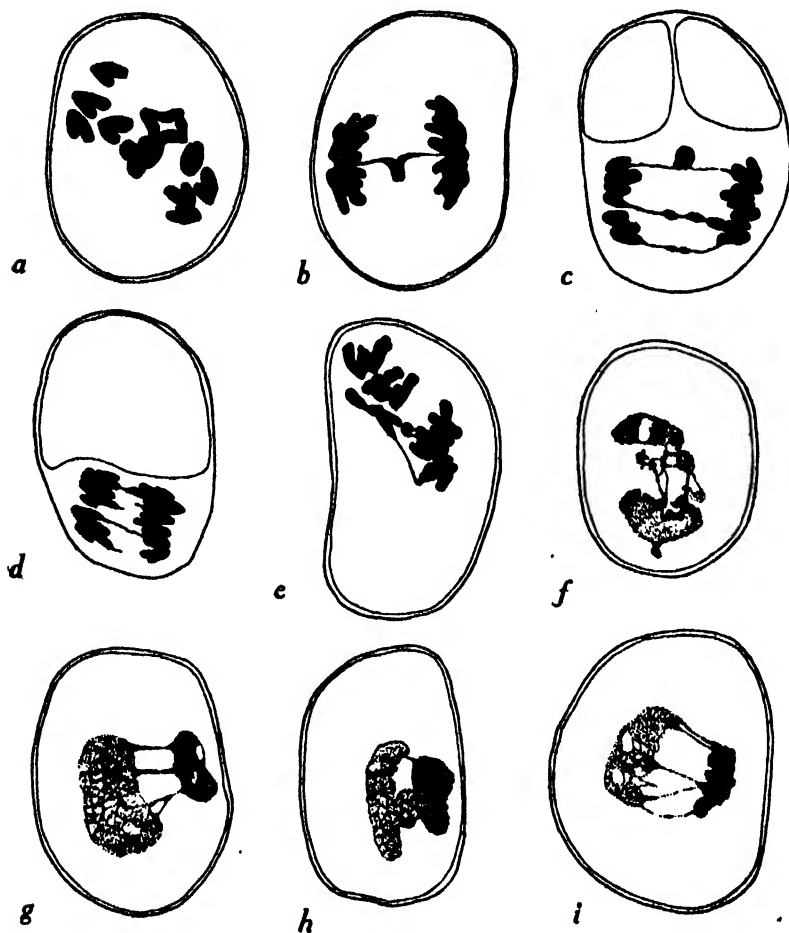


FIG. 7.—The effect of 360 r. on anaphase and telophase of P.G. mitosis. (Description in text. *a*, 2 hr.; *b*, 3 hr.; *c*, *d*, *f*, 5 hr.; *i*, 7 hr., after radiation.) $\times 1200$.

of the chromosomes within the cell, it does not interfere with the metabolism and organising processes of the cytoplasm, which proceed unhindered. The disturbance in synchronisation between the activities of cytoplasm and chromosomes must rather be attributed to the influence of radiation on the chromosomes, probably genes alone. Thus prolonged congression, lack of chromosome orientation, precocious or delayed separation of daughter chromosomes, suppression, or abnormal spindle

development, must be considered as manifestations of this disturbed synchronisation.

The order of appearance, duration, and degree of manifestation of the various primary effects following radiation with 360 r. and 90 r. is given in Table VI.

TABLE VI.—THE TIME OF APPEARANCE, GREATEST MANIFESTATION, AND DISAPPEARANCE OF PRIMARY RADIATION EFFECTS.

Radiation Effects.	Time of Appearance in Hr.		Time of Greatest Manifestation.		Time of Disappearance.	
	360 r.	90 r.	360 r.	90 r.	360 r.	90 r.
Lack of orientation of chromosomes .	1	3	3	3	12	9
Prolonged congression of chromosomes .	2	3	4-5	3	48	9
Over-spiraliation .	3	7-8	5	..	12	12
Under-spiraliation .	5	5	7	..
De-spiraliation of the telomere region .	3	7	..
De-spiraliation of the centromere region .	3
Delayed division of centromeres .	3	5	5	..	7	..
Stickiness of telomeres	2	7	7	7	48	..
Stickiness of chromosomes and chromatids .	3	7	5	7	12	..
Altered orientation of spindle .	7	24	..
Incomplete spindle .	5	7	5	..	24	12
Abnormal vacuolation	12	24	..

5. THE EFFECT OF RADIATION ON THE DIFFERENTIATION OF POLLEN GRAINS.

The two nuclei which lie after the completion of P.G. mitosis at opposite sides of the shortest axis of the P.G., differ in size and staining capacity. While the nucleus nearer the heavy wall is deeply stained with Feulgen's stain (generative or sperm-mother nucleus), the other is larger and slightly stained (vegetative or tube nucleus). The difference in staining indicates a difference in the amount of nucleic acid present in the two nuclei. Suita (1937) was the first to notice that the sperm nucleus is very rich in nucleic acid, and that there is a "remarkable decrease in the thymonucleic acid" in the vegetative nucleus of *Crinum*.

The factor or factors which are responsible for the initial differentiation of the two nuclei are not known. By analysing their structure and behaviour during the later stages of P.G. mitosis it is possible, however, to throw some light on the causes underlying differentiation. The nucleus next to the thicker wall is one which is always overcharged with nucleic acid and differentiates into a sperm nucleus. The distance between the equatorial plate and pole which the chromosomes of the generative nucleus travel through is shorter than that between the equatorial plate and the other pole to which chromosomes of the future vegetative nucleus move. It was found that the telophase stage of the generative nucleus is slightly in advance of that of the vegetative nucleus, which suggests that there might be a causal connection between position of nucleus and nucleic acid charge; it may be even considered as the initial step of differentiation. It was seen that the generative nucleus is enclosed by a thin, temporary wall at the end of P.G. mitosis. Between the wall and the nucleus a small amount of cytoplasm is included (Sax, 1935). The wall formation around the telophase chromosome group of the generative nucleus inhibits any substantial increase in the volume of this nucleus, which is conditioned by the uncoiling and despiralisation of the chromosomes. On the other hand, there is no wall around the vegetative nucleus, consequently it can expand unhindered and the chromosomes despiralise. Feulgen's staining indicates that nucleic acid is not only retained, but that it is increased in the chromosomes of the generative nucleus. It is probable that the nucleic acid which is released by the chromosomes of vegetative nucleus may be utilised by the chromosomes of the generative nucleus. It seems that the initial differences between the generative and vegetative nuclei is conditioned by the axis of division (Sax and Hustedt, 1936). However, another alternative is possible—that differentiation may be determined by the relative sizes of the cells cut off within the P.G. after mitosis. Thus the amount of nucleic acid per unit volume of nucleus is the primary factor which decides whether a particular nucleus is going to be a generative nucleus (Thomas, unpublished).

The nucleic acid charge is completed 3 days after P.G. mitosis, and this is followed by further differentiation. The generative nucleus gradually elongates, and when the length is about 4–6 times that of the width, a cleavage furrow develops in the middle, first noticed by Anderson and Sax (1934) (fig. 8, *c*). P.G.s were encountered which showed the process of unfolding of the generative nucleus along the cleavage furrow (fig. 8, *d*). The unfolding is probably due to partial uncoiling and sorting out of the closely packed chromosomes. While this morphological

change takes place Feulgen's staining shows that the amount of nucleic acid remains the same. At the end of differentiation the generative nucleus appears as a long, crescent-shaped and deeply stained body,



FIG. 8.—Various types of P.G.s, radiated with 360 r. 8 days before dehiscing. (Description in text.) $\times 1200$.

which, owing to its length, usually twists within the P.G. (Pl. I, E and F). Differentiation is complete 4–5 days after P.G. mitosis.

It is known that when P.G.s are X-rayed before the P.G. mitosis, breaks (B' and B'') are induced in the chromosomes, which are usually followed by reunion of various kinds, and results in the formation of genetically unbalanced generative or vegetative nucleus or both. The two nuclei, however, being enclosed within the same cytoplasm, there is no loss of genetic material in the P.G. in a quantitative sense; the X-ray-induced genic unbalance does not lead to degeneration and to the death of the whole cell. Because the P.G. is a closed system in which all

acentrics are present (in micronuclei), it is possible to analyse the consequences of X-ray-induced genic unbalance on nuclear differentiation and P.G. germination.

Mature P.G.s, which had been rayed with 360 r. 8 days before, show a great array of variation not only in respect of the differentiation of the generative nucleus but of the number and size of micronuclei. Table VII shows the incidence of the four most frequent types found in P.G.s after radiation.

TABLE VII.—THE VARIOUS TYPES FOUND IN 457 POLLEN GRAINS TREATED WITH 360 R, 8 DAYS BEFORE DEHISCING (PLANT 360/80), SHOWING A POSITIVE CORRELATION BETWEEN PARTIAL OR COMPLETE SUPPRESSION OF DIFFERENTIATION AND PRESENCE OF MICRONUCLEI.

Micronucleus.	P.G. Differentiation.	
	Normal.	Suppressed.
Absent . .	36.2 per cent.	3.2 per cent.
Present . .	13.1 per cent.	47.5 per cent.

It can be seen that almost half of the 457 P.G.s contain a differentiated generative nucleus; in the other half differentiation is suppressed. Our data show that there are micronuclei in most of the P.G.s in which differentiation is suppressed; only 3.2 per cent. P.G.s were encountered in which, though micronuclei were absent, differentiation was suppressed. The micronuclei are of small size and represent one or more acentrics or "minutes" (fig. 8, *a, f, g, h*). Occasionally chromosome structure or the initial shape of the acentric fragment could be seen (fig. 8, *e, l*). In several P.G.s "supernumerary nuclei" were found (fig. 8, *i*; Pl. I, G), which may be considered as "oversized" micronuclei. The vegetative nucleus proper, could not be identified in several P.G.s because of the large size of the supernumerary nucleus. Such nuclei can be formed in P.G.s through irregular chromosome segregation, which is proved by the fact that the frequency of P.G.s with supernumerary nuclei is higher when they had been rayed 3 hr. before P.G. mitosis than when the raying took place 24 hr. before division. The frequencies of P.G.s with supernumeraries were found to be 12.3 per cent. and 3.2 per cent. respectively.

In the cytoplasm of the P.G. the small, spherical micronuclei are distributed at random. They may differ in size (fig. 8, *f, g, h, i, l*; Pl. I, H),

and may also differ in staining capacity. The small micronuclei are usually well stained. It was observed that in P.G.s which were fixed 10 hr. after the completion of mitosis, acentrics always stained deeply with Feulgen's stain if they lay near the telophase chromosome group of the generative nucleus. Owing to the fact that acentrics may be left anywhere in the cytoplasm after the disappearance of the nuclear membrane, their position cannot be taken as a criterion of origin. Our observations suggest that the nucleic acid charge of acentrics is determined solely by their position at late telephase or soon after the completion of P.G. mitosis. It may be the case that when the thin temporary wall of generative nucleus surrounds the acentrics, they also acquire a heavy nucleic acid charge, similar to that of the chromosomes of the generative nucleus, irrespective of their origin.

The frequency of P.G.s showing micronuclei depends on the dose; when P.G.s were rayed with 360 r. 8 days before anther dehiscence 60.6 per cent. showed micronuclei, while with 90 r. only 28.2 per cent. showed micronuclei. The mean number of micronuclei per P.G. was 2.7 and 0.8 respectively. Sax (1938) reported that after 150 r. 22.0 per cent. of the pollen grains show micronuclei.

An analysis was made to determine if there is any relationship between the presence of micronuclei and the suppression of differentiation. Differentiation of generative nucleus is not prevented when only the small and deeply stained micronuclei are present in the P.G. (Pl. I, E). Furthermore, it was observed that differentiation is independent of the size and number of such micronuclei (compare fig. 8, *b* and *h*). On the other hand, in the presence of a large supernumerary nucleus differentiation is suppressed (Pl. I, G). Only 3 P.G.s were encountered amongst the 457 analysed, in which a supernumerary, or oversized micronucleus, and a well-differentiated sperm nucleus were found together. It is probable that differentiation in the generative nucleus is correlated with the nucleic acid charge, because when a large acentric or whole chromosome is lost from the sperm nucleus, the significant decrease in nucleic acid within the P.G. is followed by the failure of differentiation. If, however, the loss is made good by an increased charge in the micronuclei, differentiation of the generative nucleus is completed.

Amongst the 457 irradiated P.G.s, 2 were found which showed mitosis at the time of anther dehiscence. Division of these P.G.s has been delayed at least 7 days longer than in sister P.G.s. The two cells have shown (1) precocious separation of sister chromatids at metaphase, and (2) a very high number of minute fragments or acentrics, grouped together at one place, previously occupied by the resting nucleus (fig. 8, *j*).

It is supposed that these cells may have been reverted from prophase to resting stage. The long delay in P.G. mitosis must be due to the great number of induced structural changes. It is not improbable that a complete suppression of P.G. mitosis may be induced by radiation. In our experiments, however, no P.G.s were seen with one nucleus at de-hiscence, which would be the only criterion of complete suppression of P.G. division.

6. THE EFFECT OF RADIATION ON POLLEN GRAIN GERMINATION.

Germination usually begins 1 hr. after sowing the pollen of *Tradescantia* on artificial culture medium (Swanson, 1940 *a*). Eigsti (1940) reported that germination may start within 15 minutes after sowing.

The beginning of germination is characterised by various changes within the P.G. The vegetative nucleus may degenerate to such an extent that, at 8 hr. after sowing, only a small, lightly stained body of various shape lying in the cytoplasm can be identified as the remnant of the vegetative nucleus (fig. 9, *a*, *b*). Instances were also found where the vegetative nucleus enters the P.T. as an oblong or ball-shaped compact body (fig. 9, *k*; Pl. I, D). In other P.G.s this nucleus is transformed into a long, thin thread, which can stretch far into the P.T. (fig. 9, *g*, *l*; Pl. I, C). This amoeboid behaviour is due to the fluid content of the nucleus and to the strong cytoplasmic streaming directed towards the distal end of the tube. The content of the long thread-like nucleus may flow out into the cytoplasm at one end.

It is concluded, that the function of the vegetative nucleus is to start (1) cytoplasmic streaming and (2) the outgrowth of the tube. X-ray-induced changes within the vegetative nucleus may interfere or arrest either of these processes. The suppression of cytoplasmic streaming (fig. 9, *j*, *m*) is more frequent than a complete arrest of P.G. growth (fig. 9, *c*).

In the generative nucleus there is no proper prophase; the chromosomes are already split, fully organised, spiralised, and coiled to an extreme degree. After the disappearance of the nuclear membrane they fall apart and their relic coils uncoil (Pl. I, A). If germination is delayed the individual chromosomes may separate while the nucleus is still in the P.G. (Pl. I, D). Normally the division takes place in the tube. Six P.G.s, each of which had a very short tube, were found amongst 341 in which the division of the generative nucleus had already begun in the P.G. In the generative nucleus, after the disappearance of the nuclear membrane, a deeply stained granule is seen, which can easily be mistaken

for an acentric fragment (Pl. I, B, C). The function of this granule is not known (Anderson and Sax, 1934; Eigsti, 1940).

The wall of the P.G. is much thinner at the ends (germ-pores), the

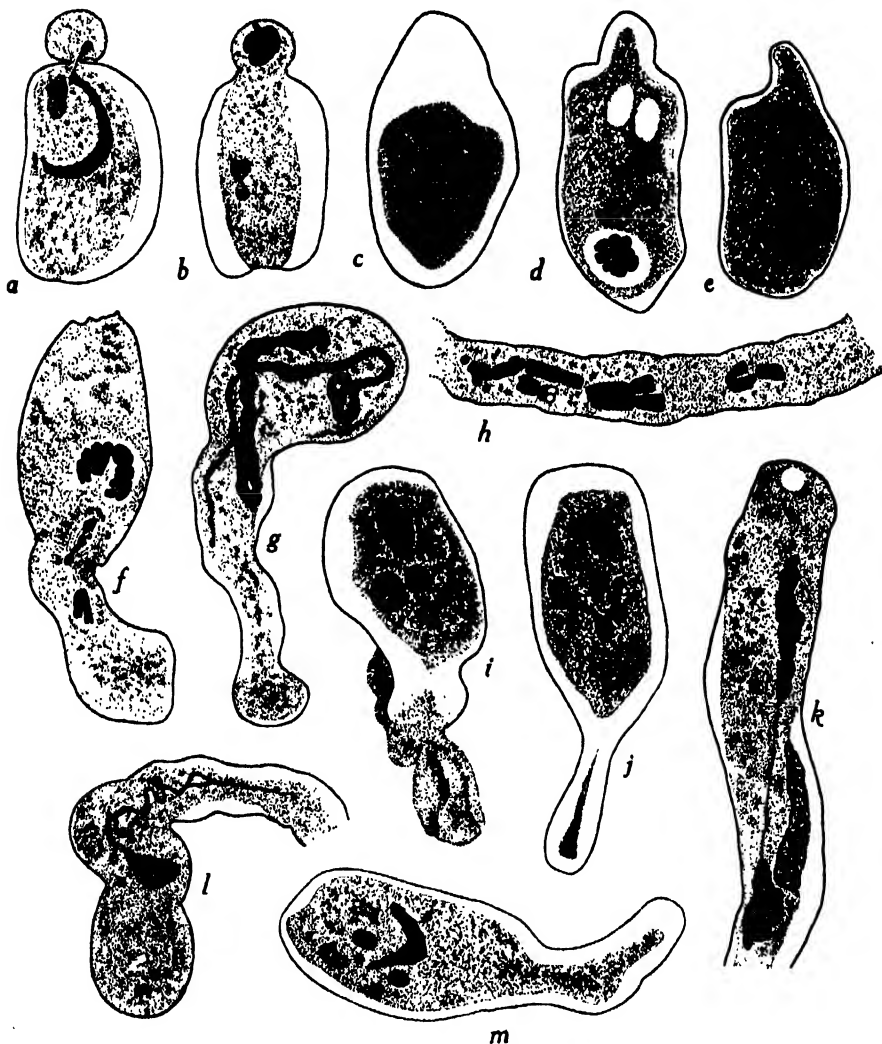


FIG. 9.—Germination of normal and radiated P.G.s. (Description in text.) $\times 1200$.

outgrowth of the tube taking place in these regions. The thin membrane of germ-pores breaks and the cytoplasm of P.G. is forced through the opening, and form a bulge. If the generative nucleus lies near, a part of it may be carried away with the cytoplasmic streaming (fig. 9, a).

Later, when the opening and the cytoplasmic bulge increases, the whole generative nucleus may be moved into it (fig. 9, *b*). Sometimes a whole chromosome or chromosomes may be carried away from the generative nucleus (fig. 9, *f*). The centromeres of such chromosomes often turned into a direction opposite to that of streaming, indicating that their movement is passive (Swanson, 1940 *b*). When the generative and vegetative nuclei are far away from the developing P.T. their entry into the tube may be delayed. The sequence of entry is determined solely by the position of the two nuclei.

It was found that, on artificial culture medium, occasionally two tubes grow simultaneously from the opposite ends of one P.G. Usually one of the tubes grows faster than the other; only a few P.G.s were seen with two equally long P.G.s.

P.G.s were radiated before and after mitosis. The germination frequency of P.G.s, when they have been rayed with 90 and 360 r. after P.G. mitosis, is 93.8 per cent. and 91.1 per cent. respectively (Table VIII).

TABLE VIII.—GERMINATION OF POLLEN GRAINS X-RAYED AT ANTHER DEHISCENCE.

Dose.	Plant.	Total No. of Pollen Grains.	Per cent. of Germination.
90 r.	90/71	191	94.1
	90/72	227	90.0
	90/73	183	97.1
	Total .	601	93.8
360 r.	360/91	240	89.7
	360/97	213	94.3
	360/98	236	89.1
	Total .	689	91.1
0	..	600 *	96.2

* Data obtained from three plants; 200 pollen grains from each plant.

Our data show that germination is not affected when P.G.s are rayed after P.G. mitosis, and corroborate the findings of Poddubnaja (1936) and Newcombe (1942). If radiation occurred 6–8 days after P.G. mitosis, or just before sowing, the chromosomes of sperm nucleus exhibit (1) stickiness at pre-metaphase stages of the P.T. division (fig. 9, *k*), and (2) structural changes, such as breaks (*B'* and *B''*), reunions, and acentric

fragments (fig. 9, *h*). It can be seen that while radiation is effective on the chromosomes of the generative nucleus, at the same time it is ineffective on the vegetative nucleus. Germination of P.G.s is not prevented even when a very high dose (10,000–30,000 r., Poddubnaja, 1936) is used. One may assume that the release of the total, or almost total, nucleic acid charge of the chromosomes may alter the organisation of the vegetative nucleus to such a degree that it becomes radio-resistant.

On the other hand, germination of those P.G.s which are rayed before

TABLE IX.—GERMINATION OF POLLEN GRAINS X-RAYED BEFORE P.G. MITOSIS.

Dose.	Time of Radiation in Days before Dehiscence.	Per cent. of Germinated Pollen Grains.	Total No. of Pollen Grains.
90 r.	8	45.1	221
	9	38.9	372
	10	22.3	245
360 r.	8	17.6 *	435
	9	11.8	271
	10	8.7	223
800 r.†	8	2.5	Not given
	10	1.5	"

* Mean from three different plants.

† Data obtained by Newcombe (1942).

P.G. mitosis is greatly affected (Table IX). The percentages of germinating and non-germinating P.G.s is given in Table X. Germination was classified as normal when there was a long, well-developed tube, in which the chromosomes could be clearly distinguished. It was observed that P.G.s which germinated normally had a differentiated generative nucleus. About one-third of these P.G.s had, furthermore, one or two small, deeply stained micronuclei. The tubes of those P.G.s which had supernumerary nuclei or lightly stained micronuclei, or an undersized vegetative nucleus, either did not grow at all (fig. 9, *c*), or grew very slowly (fig. 9, *m*), or burst (fig. 9, *i*). The germination of P.G.s without differentiated generative nucleus or with supernumerary nuclei were also arrested (fig. 9, *j*). The cytoplasm of such P.G.s becomes vacuolated (fig. 9, *d*), and stains deeply with lacmoid or carmine (fig. 9, *e*). It was found that the cytoplasm in the short P.T.s may be completely isolated from that within the P.G. The extreme condensation of cytoplasm in

non-germinating P.G.s (fig. 9, *c*, *e*) indicates that the cytoplasmic movement is suppressed. The behaviour of radiated P.G.s suggests that the vegetative nucleus is a centre of a mechanism which conditions the response between the substrate (stigma or culture medium) and P.G. and initiates the first stage of germination.

Several morphologically normal, non-germinating P.G.s were encountered amongst those which were radiated before mitosis. It was seen that out of 36.2 per cent. with a differentiated generative nucleus only 13.9 per cent. P.G. germinated (Tables VII and X). The data suggest that in 22.3 per cent. of non-germinating P.G.s the X-ray-induced unbalance is either genic in nature or that it is due to interchanges. Owing to the high percentage it is reasonable to assume that the failure of germination of some morphologically normal P.G.s may be due to lethal gene-mutation. Our data clearly indicate that the degree of fertility of P.G.s when the radiation is carried out before P.G. mitosis, cannot be measured directly by the frequency of morphologically normal P.G.s in which micronuclei are absent. P.G. fertility was calculated by Sax (1938) on this basis. He estimated that 78 per cent. of P.G.s when rayed with 150 r. 8 days before anther dehiscence are "fertile" because they have no micronuclei. When the necessary correction is made, the frequency of actually germinating P.G.s would not be more than 34 per cent. in his experiment, which is in good agreement with our estimate given in Table X.

TABLE X.—TYPES OF POLLEN GRAINS IN PERCENTAGE AFTER 36-HOURS' GERMINATION, TREATED WITH 360 R. 8 DAYS BEFORE DEHISCENCE. (659 POLLEN GRAINS.)

	Normal.	Short Tube.*		No Tube.
		Not Burst.	Burst.	
Without Micronuclei .	13.9	9.12	6.4	15.1
With Micronuclei .	5.2			50.1
Total .	19.1	15.6		65.2

* Micronuclei are not scorable.

7. DISCUSSION.

Radiation of P.G.s throws light on two important problems: the degree and type of differentiation (1) within chromosomes and (2) between nuclei

1. A great wealth of data has been accumulated during the last few years which shows that all the later effects of radiation can be classified as due to alterations in the organisation of chromosomes. However, suppression of the P.G. mitosis, detectable only by a quantitative analysis, is actually the result of qualitative changes, induced in those processes which are introductory to the onset of prophase, and hence primarily concerned with chromosome organisation. It is postulated that previous to prophase several changes must take place within the "resting" nucleus. These changes are related to gene-multiplication, nucleic acid polymerisation, and molecular spiralisation. The arrest of any of these processes results either in the suppression of division or in the prolongation of "resting" stage.

While the "resting-stage-prolonging" or "division-suppressing" effect can be detected only by a quantitative analysis, the effect of radiation on the metaphase chromosomes is shown by qualitative as well as quantitative changes. Both prolonged congression and stickiness are believed to be due to disturbance in nucleic acid synthesis; the amount of nucleic acid is increased and deposited on the chromosomes more rapidly than under normal conditions (Darlington, 1942).

It was, furthermore, observed that two regions of the chromosome, namely the telomere and the heterochromatic region adjacent to the centromere, exhibit an increased sensitiveness to radiation. Stickiness is induced more frequently in the telomere than in the intercalary regions. The higher radio-sensitiveness of heterochromatin is expressed by a higher frequency of breakage (Muller, 1941) and by delayed spiralisation, when compared with that of the euchromatic regions: and this reveals a different structural organisation, specific to these particular regions.

2. Nuclear division is preceded by an increase in the amount of nucleic acid within the cell and nucleus, which is utilised in building up the structure of the chromosomes (Caspersson, 1941). It is evident that P.G.s have a different chemical organisation and metabolism from that of other cells, because the chromosomes of the generative nucleus retain the nucleic acid charge between the P.G. and P.T. mitosis, and no nucleic acid is required by the vegetative nucleus for chromosome organisation. The localisation of nucleic acid and the change in the chemical metabolism of cytoplasm may be considered as adaptations of primary importance which enables the pollen to fulfill its function.

The failure of radiation in suppressing germination when differentiated P.G.s are treated, indicates that there is a fundamental change in the structure of the chromosomes of the "resting" vegetative nucleus. While chromosomes of ordinary resting nucleus have a small amount of nucleic

acid attached, chromosomes of the vegetative nucleus of P.G.s are almost completely free, and remain so throughout the cycle. It is not improbable that this difference is responsible for the failure of radiation in suppressing germination.

On the other hand, the chromosomes of the generative nucleus between the telophase of P.G. and P.T. division are heavily charged with nucleic acid. Their reduplication (splitting), which occurs not later than at or about the time of anther dehiscence (Swanson, 1940 *b*), must have taken place in the presence of the nucleic acid attachment. The behaviour of the generative nucleus shows that not only breaks and reunions can take place, but gene and chromosome reduplication are also possible in chromosomes which are heavily charged with nucleic acid.

The germination of radiated P.G.s shows that radiation does not directly kill the cell, but death always comes about through changes induced in the chromosomes. If these are structural changes, they may be identified at the succeeding division as breaks, reunions, and acentrics. Division following radiation is the usual way by which the radiation effects come into operation. Genic unbalance, a concomitant of the induced structural or gene changes, is responsible for the death of the daughter cells. The induced damage to the chromosomes may be very extensive. The derangement of the nucleic acid cycle may cause not only a long but complete suppression of division. There is also a further possibility that radiation can also induce dominant lethal-gene-mutation; thus the death of the cell after radiation may come about without division. But in all these instances the primary effect of radiation is always in the chromosomes.

8. SUMMARY.

1. In *Tradescantia* there are developmental differences within anthers, and between sister anthers of the same flower-bud.
2. The rate of P.G. mitosis depends on the phase of the development cycle.
3. The proportion of dividing P.G.s in metaphase and post-metaphase stages is the same during the different phases of the cycle. It is the same in sister anthers.
4. A 360 r. X-ray dose (at temperature of 16°–24° C.) suppresses the beginning of prophase, which reduces the rate of change from one to two nuclei in P.G.s.
5. P.G.s which are within 1 hr. of P.G. mitosis are apparently not affected by radiation.

6. The "prophase-suppressing" effect is greatest 3 hr. after radiation, and lasts 2 hr.

7. The metaphase of P.G. division is prolonged. The duration of metaphase may be increased to 3 hr. Prolongation of metaphase was found 48 hr. after radiation.

8. There is an indication that all stages of P.G. mitosis are retarded, the effect on metaphase being the most obvious.

9. The radiation effect decreases with time.

10. 90 r. X-ray dose suppresses prophase very little, and the small "metaphase-prolonging" effect comes into operation 7 hr. after radiation.

11. Prolonged congression of chromosomes induced by radiation may lead to the complete suppression of metaphase stage proper.

12. Stickiness of chromosomes and chromatids is attributed to an excess deposit of non-polymerised nucleic acid on the chromosome under the influence of radiation.

13. The nucleic acid charge of the generative nucleus is retained after P.G. mitosis; the chromosomes of vegetative nucleus lose most, if not all, their nucleic acid attachment.

14. The nucleic acid charge of acentrics or micronuclei are determined solely by their position at the end of P.G. mitosis. Acentrics lying next to the generative nucleus are heavily charged, irrespective of their origin.

15. Differentiation of sperm nucleus is determined by the amount of localised nucleic acid in the chromosomes and cytoplasm.

16. P.G. mitosis can be delayed for long or even may be completely suppressed by radiation.

17. X-ray-induced genic unbalance within the generative or vegetative nucleus suppresses differentiation, but does not lead to the death of the P.G. itself.

18. Normal germination of P.G. can take place only (a) if there is a differentiated generative nucleus, and (b) if such micronuclei as may be present are not of the large (supernumerary nucleus) or lightly stained type.

19. Abnormal germination occurs when (a) generative nucleus is undifferentiated, (b) vegetative nucleus is undersized, (c) and several lightly stained micronuclei are present.

20. Germination is completely suppressed when the vegetative nucleus is undersized, or when it is accompanied by supernumerary nucleus.

21. Failure of germination of morphologically normal P.G.s suggests that radiation induced either gene-mutation or interchanges in the chromosomes of vegetative nucleus.

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P. C. KOLLER

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II. DESCRIPTION OF PLATE.

- A. P.T. showing the six chromosomes. × 1200.
- B. P.T. showing two separate groups of chromosomes of the sperm. The small nuclear granule is clearly visible. × 1200.
- C. P.T. showing the amoeboid-like vegetative nucleus, the nuclear granule, and the chromosomes of sperm nucleus. × 1200.
- D. Delayed germination; the nuclear membrane of sperm nucleus disappeared within the P.G. × 1200.
- E. P.G. with differentiated sperm nucleus in the presence of micronucleus. × 800.
- F. Two P.G.s with differentiated sperm nucleus and micronuclei of varying sizes. The third large P.G. is diploid. × 800.
- G. P.G. showing supernumerary nucleus. There is no differentiation. × 800.
- H. P.G.s containing micronuclei. The differentiation of the sperm nucleus is suppressed. × 800.

(Issued separately April 23, 1943.)

XXX.—Study of an Introduced North American Freshwater Mollusc, *Stagnicola catascopium* (Say). By D. Keith McE. Kevan, B.Sc., Zoology Department, University of Edinburgh. Communicated by Professor J. RITCHIE, M.A., D.Sc. (With Ten Graphs and Twenty Text-figures.)

(MS. received November 14, 1942. Revised MS. received April 7, 1943.
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CONTENTS.

	PAGE
I. INTRODUCTION	430
II. GENERAL NOTES ON ANATOMY AND ECOLOGY OF LEITH SPECIMENS .	431
III. LIFE-HISTORY	435
IV. VARIATION OF SHELL IN LEITH SPECIMENS.	438
V. VARIATION OF SHELL IN AMERICAN SPECIMENS	453
VI. POSSIBLE CAUSES OF VARIATION.	454
VII. EXPERIMENTS IN AQUARIA	456
VIII. SUMMARY AND CONCLUSIONS	459
IX. ACKNOWLEDGMENTS	460
X. REFERENCES TO LITERATURE	460

I. INTRODUCTION.

IN July and August 1929 the writer's father, Mr D. K. Kevan, found large numbers of a species of *Lymnæa*, cleaned out of a warm engine-pond in a timber yard in Leith. These snails showed much variation in the form of the shell, and this made identification difficult.

In July and August 1940 the writer revisited the locality, collected a considerable number of living snails from the pond in question, and eventually established that they belonged to the common North American species, *Lymnæa (Stagnicola) catascopium*, Say.

Since this species has not previously been recorded from Britain a brief description of the differences between it and native British *Lymnæids* resembling it has been given in a preliminary paper by Kevan (1941, p. 279), but, for a full description of *S. catascopium*, readers are referred to Baker (1911).

Jeffreys (1862, p. 115) refers to the North American species *Lymnæa cornea* (no authority) as having been found in the west of Ireland, though he discredits the record, and *L. cornea* Valenciennes is a synonym of *L. catascopium*, Say.

The natural distribution and ecology of the species are fully discussed by Baker (1911, 1928).

The species appears to have been introduced to the timber yard at Leith with logs of birch and elm imported from Eastern Canada, for these on arrival are immediately floated in the engine-pond. The snails, at any rate juvenile forms, could travel quite safely hidden in the cracks to which these logs are subject, although the possibility of their having arrived as *ova* must not be overlooked. Boycott (1936, p. 123) states that the egg-masses of water-snails are resistant and can be moved about from one sort of water to another with impunity.

II. GENERAL NOTES ON ANATOMY AND ECOLOGY OF LEITH SPECIMENS.

Shell.—Leith specimens are very rarely thick and solid, but usually, not invariably, rather thin; about 2 per cent. only dark chestnut colour, remainder mostly very pale; spiral sculpture always fine; peristome, normally, not much thickened or edged with chestnut. The spiral malleations associated with *S. palustris* (Müll), with which species it might otherwise be confused, are absent. The range of variation of shell-form is much greater than that given by Baker (1911), and it is noteworthy that this variation occurs in one and the same *locus*. This will be referred to in greater detail later. The size was never as large as that of some of those quoted by Baker (1911), seldom exceeding 16 mm. in length, an average adult being about 13 mm.

Animal (fig. 1).—All were blackish, with white flecking, this flecking covering even the sole of the foot, unlike the British *palustris* in which the sole of the foot is unspotted. Tentacles rather more pointed than in *palustris*, and slightly curved.

Radula.—It may be noted that the sub-genus *Stagnicola* is distin-



FIG. 1.—*Stagnicola catascopium*. ($\times 3\frac{1}{2}$.)

guished from other sub-genera of the Lymnæidæ by the lateral teeth of the radula being bi-cuspid; intermediate teeth tri-cuspid. Baker's figure is given for reference (fig. 5).

The dimensions of the 1st lateral teeth of about the 59th row were

Height of base, 20.75 μ .	Width of base, 20.75 μ .
Height of tooth, 31.13 μ .	Width of tooth, 18.87 μ .

It was found that the radula did not seem to agree very closely in some respects with the formula, but this formula, as indicated by Baker (1911), is liable to considerable variation, and in any case is not easy to determine accurately. A specimen of the radula of *S. catascopium* in the Gwatkin Collection of radulæ (Department of Zoology, Edinburgh University) agreed fairly well with Baker's description, as did some of those from Leith. In the Leith specimens examined, the chief differences were found in the transition teeth, and there were fewer cusps to the marginals. Leith radulæ contained about 105 rows, the formula usually being

$$\frac{20}{5-6} + \frac{5}{3-4} + \frac{9}{2} + \frac{1}{1} + \frac{9}{2} + \frac{5}{3-4} + \frac{20}{5-6} \quad (34-1-34),$$

although some had 21 marginals (35-1-35) and others 10 laterals (35-1-35).

Genitalia (figs. 2, 3, and 4).—The sub-generic genital characters of *Stagnicola* are: prostate long-ovate; penis-sheath $\frac{3}{4}$ of, to as long as preputium; sarcobelum, a small muscular ridge (fig. 3). As compared with *S. palustris*, the spermatheca of *S. catascopium* is round and not pyriform. The writer's figures of variation of musculature of the male organ in Leith specimens (fig. 4) show even more variation than Baker's, particularly example D, with its complex series of connective muscles. Hardly any two specimens were alike, but the retractor muscles were more or less uniform, as Baker (1911) states.

Ecology.—Habitat: The habitat is a rectangular concrete-walled pond, 72 ft. long, 36 ft. broad, and $6\frac{1}{2}$ – $7\frac{1}{2}$ ft. deep (depending on water-level), converted from engine-pit of old sawmill in 1900, when new mill was constructed. Source of water: natural spring (principally), rain-water and Edinburgh tap-water. Constant circulation is maintained over a condenser. The spring-water is received at the condenser, and this, together with the pond-water in circulation, passes back to the pond in a heated condition by way of a shower helping to cool the water. In addition to the pipe leading to the condenser there are two overflow pipes, in the inflow parts of which large numbers of healthy snails live.

The high temperature of the pond, which originally suggested the investigation, is normally 27° C. The water from the condenser does

not flow at night, or during the lunch-hour, so that there must be a slight drop at night—particularly during the winter week-ends. The temperature is uniform throughout the pond.

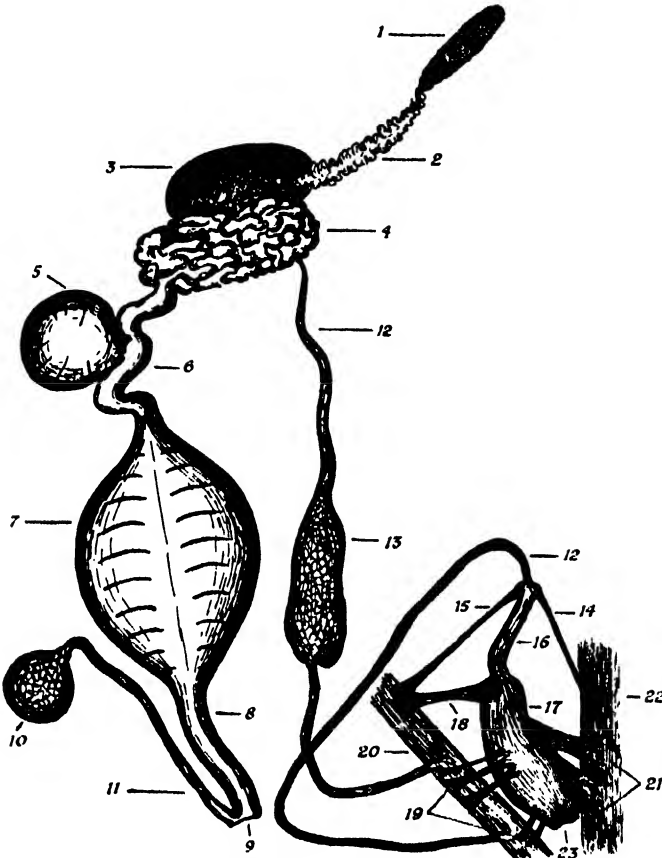


FIG. 2.—Dissection of Genitalia. ($\times 6$.)

- | | | |
|-------------------------|------------------------|--------------------------------------|
| 1. Ovotestis. | 9. Female aperture. | 17. Preputium. |
| 2. Hermaphrodite duct. | 10. Spermatheca. | 18. Preputium retractor. |
| 3. Albumen gland. | 11. Spermathecal duct. | 19. Posterior preputium protractors. |
| 4. Common genital duct. | 12. Vas deferens. | 20. Columella muscle. |
| 5. Nidamental gland. | 13. Prostate gland. | 21. Anterior preputium protractors. |
| 6. Oviduct. | 14. Penial nerve. | 22. Dorsal body-wall. |
| 7. Uterus. | 15. Penial retractor. | 23. Male aperture. |
| 8. Vagina. | 16. Penis sheath. | |

(*N.B.*—This specimen had more than the usual number of preputium protractors.)

The pH is approximately 7.5, the same as that of Edinburgh tap-water. The pond is emptied, cleaned, and its walls lime-washed annually.

Numbers and Distribution: The snails show a definite preference for the walls of the pond in the upper foot of water, probably because algal

food is more abundant. They also show an inclination to congregate in corners and on ledges (e.g. over 2000 specimens of all ages were taken from 18 ft. of ledge). In Leith, *S. catascopium* was found abundantly

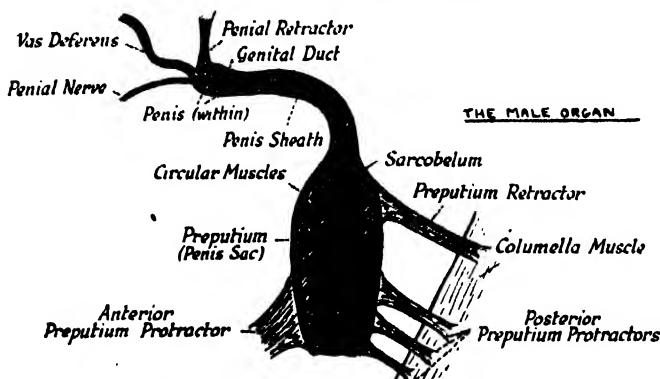


FIG. 3.—The Male Organ. ($\times 7\frac{1}{2}$.)

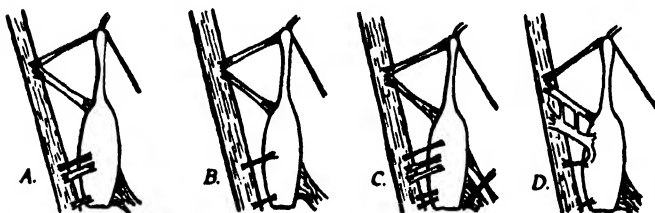


FIG. 4.—Variation in Musculature of the Male Organs.



FIG. 5.—Part of Radula. (After Baker.)

at all depths down to $7\frac{1}{2}$ ft., the greatest depth of the pond. A census of numbers gave:

1st foot,	105 snails per sq. ft.
3rd „	54 „ „
7th „	36 „ „

A gradual decrease in density of population occurs, therefore, with increase of depth of water, possibly corresponding with a decrease in algal food due to diminishing light. As the water is constantly circulating, there is probably as much oxygen at the bottom as at the surface. The average density of population was estimated at 54 per square foot of concrete, which yields 221,616, or roughly a quarter of a million snails in the pond.

Food: A number of stomachs were examined. The main diet of the snails is undoubtedly the abundant supply of unicellular algæ occurring on the walls, on the surface as a scum, and in suspension. Multicellular algæ were practically non-existent, excepting for a small quantity of *Vaucheria* sp. (possibly accidental), and a thin thalloid scum-like form, probably *Prasiola* sp. In past years the pond was extensively matted with a filamentous alga, possibly *Spirogyra* sp., but this has now disappeared. Practically all the specimens dissected contained, strangely enough, the statoblasts of the Bryozoon *Plumatella repens*. One specimen had so many of these statoblasts that large numbers issued from the anus as the snail was dissected. No fragments of *Plumatella* colonies were found in the gut. That water-snails may be carnivorous is noted by Boycott (1936, p. 119), but the devouring of statoblasts of Bryozoa must be considered unusual. Ingestion may have taken place with other food from the wall of the pond, as the statoblasts were very common on the surface of the water, especially at the margins.

III. LIFE-HISTORY.

(1) *Rate of Reproduction.*—The egg-laying capacity of *S. catascopium* is relatively small in comparison with some species of *Lymnæa* (e.g. *L. (Radix) peregra*), which produces an average of 500 eggs, and may lay as many as 3000 during its lifetime (Boycott, 1936, p. 120).

The egg-masses are from 10 to 15 mm. long by 5 to 7 mm. broad, and contain from 10 to 54 eggs, averaging 25 eggs per egg-mass. The capsules in question, however, were laid in September out of the true breeding season, which is July (Baker, 1928, p. 254), and the writer would estimate 50 eggs per egg-mass to be nearer the mark for a mature snail in July.

It is interesting to note that, in one capsule, eggs with twin embryos were observed.

S. catascopium may be self-fertilised in captivity (if isolated), as is frequent among *Lymnæa* (Boycott, 1936, p. 120). The number of capsules laid by a single individual of *S. catascopium* was not determined with certainty, but it is probably only one.

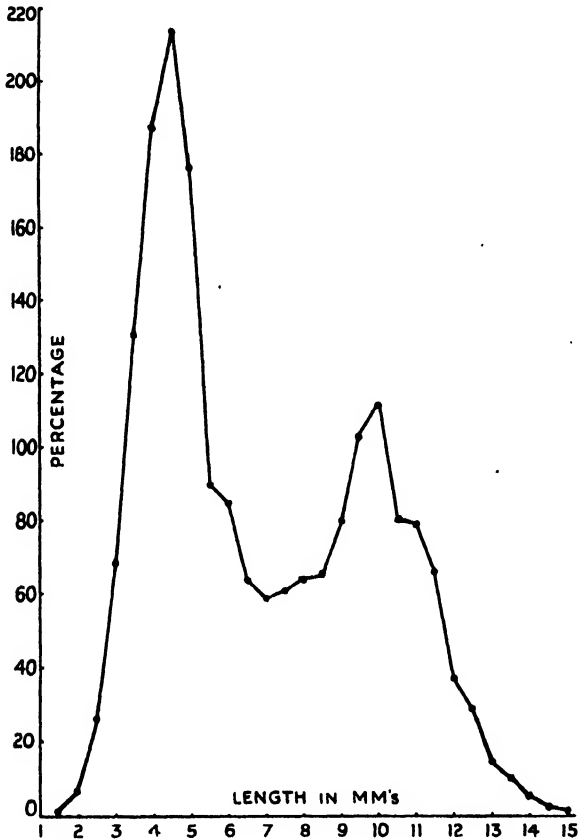
(2) *Length of Life, and Number of Broods per Annum.*—The length of life of the snails in Leith could never be more than twelve months (with the possible exception of those living in the overflow pipes), even if this species were an exception to the general rule that aquatic pulmonate mollusca live but one year or less (Boycott, 1936, p. 121). From specimens kept in aquaria, the duration of life would appear to be about one year at 27° C., but rather more at lower temperatures. *R. peregra*, in hot

summers, may go through two generations, as it may easily be induced to do in captivity (Boycott, 1936, p. 121). This is significant with regard to *S. catascopium* in the warm water of the Leith pond, as it has two main broods per year (as shown hereafter) instead of one brood. Baker (1928, p. 254) gives only one breeding period. Another possible stimulant to multiplication suggested by Boycott (1936, p. 169)—referring to Backrach and Cardot's paper (1924, *C.R. Soc. Biol.*)—is "that brief spells of daily cooling promote the breeding of *Limnaea stagnalis*." Now it has already been noted that the temperature of the pond may drop slightly each night when the warm current is shut off. 27° C. is probably about the optimum temperature for breeding. Boycott (1936, p. 169) suggests that as much as 30° C. for prolonged periods would be lethal for British species of freshwater mollusca. With regard to low temperatures, specimens of *S. catascopium* from Leith survived being frozen in solid ice for more than three days.

S. catascopium has two main broods per year in Leith, but continues to breed continuously throughout the year. This is clearly demonstrated in graph 1, with its two modes, and the trough, even at its lowest point, well above zero. Of the snails collected, over 1900 specimens of all sizes, from 1.5 mm. to 15 mm. long, were measured to the nearest half-millimetre. Length of shell was taken as being proportionate to the age—though this is not strictly true. At least some of the large specimens of 13.5 mm. and over may be the original snails which issued from the overflow pipes as immature snails, which, upon reaching maturity, started the new colony. These produced the snails of about 10 mm., which in turn produced those reaching about 4.5 mm. at the time of collection. Constant subsidiary breeding continues all the year round. The 4.5 mm. snails would be about one month to six weeks old (determined by breeding experiments), which would indicate this brood as having been produced in early July or late June 1940—about the normal breeding-time in America—while those of 10 mm. would belong to a brood produced in the spring of 1940 by the older (13.5 mm.) snails.

(3) *Mortality*.—From graph 1, a rough estimation of the rate of mortality was made. Assuming that the 10 specimens of 13.5 mm. gave rise to the 450 between 9 and 11 mm., one would expect the second brood (say between 3.5 and 5.5 mm.) to be somewhere in the region of 20,250 in number (450×45), instead of which it is a mere 800, giving less than 4 per cent. survival. There thus appears to be a 96 per cent. mortality of those which survive past the egg stage in the second brood. While accuracy in this estimation is not possible owing to the difficulty of collecting the very young individuals of the second brood, there is

undoubtedly a very heavy mortality, obviously due to the overcrowded conditions and consequent competition for the available supply of food encountered by this brood. The first brood, on the other hand, has plenty of space and plenty of food, as the algæ would reproduce in the newly



GRAPH 1.—Length and Frequency (based on 1918 shells).

cleaned pond more rapidly than the snails would be able to eat them. Thus, overcrowding seems to be the only adverse factor affecting the multiplication of the snail.

(4) *Rate of Growth*.—This would appear to vary greatly with the individual snail, specimens bred at 27° C. from the same group of egg-capsules in the same container varying, after six weeks, between 4 and 9 mm. in length. The age of maturity might likewise vary, although size is probably not the main factor determining maturity. Growth is, of course, much slower at lower temperatures. At room temperature (about 15° C.) few individuals reached 4 mm. in the same period of time.

IV. VARIATION OF SHELL IN LEITH SPECIMENS.

(1) *General*.—The amount of variation in the form of the shell of this species, as found at Leith, is phenomenal (*vide* photograph, Kevan, 1941, p. 303). This variation is all the more remarkable because of the uniform environment, for even a variable snail like *R. peregra* shows a uniform trend in variation in any one environment. Not only is the variation displayed in the proportionate length to breadth of shell or to height of aperture, height of aperture to breadth of shell or width of aperture, etc., but also in the form of the lip, which ranges from perfectly normal to greatly flared, or through various stages of deflection and reflexion until it doubles completely round on itself and forms a heavy white rim; and further in the texture of the body-whorl, which may be smooth, striated, varicated, greatly dilated, or even heavily ribbed. None of these variations is in the nature of a monstrosity such as is referred to by Boycott and Diver (1930). The only monster found was a "scalarescent" form (one specimen), not unlike that figured by Baker (1911, pl. xl, fig. 31). No sinistral shell was found. All the various forms illustrated by Baker (1911) occur living together in Leith, with the exception of those figured by him at pl. xl, figs. 24, 32–35; pl. xli, figs. 1 and 2; pl. xlvii, fig. 10. Many others, which he has not illustrated, are also found in Leith (see the accompanying figures). The writer recognises 16 different types of shell, all of which, however, grade into one another by intermediates, but any one shell can usually be roughly classified under these types. Many show more than one character.

1. *The "Normal" Type*. Figs. 6 and 6A.

This type corresponds more or less with the normal American type. It has fine, though distinct, striæ, and a spire of moderate length. Cf. Baker (1911), pl. xl, figs. 7, 9, 10, 11, 13, 17, 18, etc.

2. *Short-spined Type*. Fig. 7.

This resembles the var. *pinguis* (Say) in being short and stumpy. Cf. Baker (1911), pl. xl, figs. 10, 22, 27, and 28.

3. *Narrow Type*. Fig. 8.

Resembles normal type, except in being unusually narrow, and often with striæ very obscure. Cf. Baker (1911), pl. xl, fig. 6.

4. *Tall-spined Type*. Fig. 9.

Spire much taller than normal, sometimes even with extra whorl, although this is unusual. The height of the aperture is correspondingly reduced. Cf. Baker (1911), pl. xl, fig. 19.

5. *Auriculate Type*. Fig. 10.

Aperture large and swollen. Spire usually shorter and sharp. Otherwise normal. Cf. Baker (1928), pl. xvii, fig. 28.

VARIOUS SHELL TYPES. ($\times 2\frac{1}{2}$.)



FIG. 6.—"Normal" Type.



FIG. 6A.—Basal End-elevation of "Normal" Type.



FIG. 7.—Varicated Type. (*Pinguis*-like form.)



FIG. 7A.—Basal End-elevation of Varicated Type.



FIG. 8.—Narrow (Sub-fusiform) Type.



FIG. 9.—Tall-spined Type. (N.B.—Extra whorl.)



FIG. 10.—Auriculate Type.

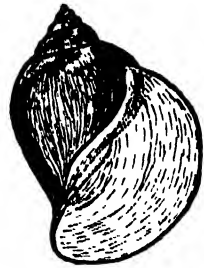


FIG. 11.—Dilated Type, Stage 1.



FIG. 11A.—Basal End-elevation of Dilated Type, Stage 1.



FIG. 12.—Dilated Type, Stage 2.



FIG. 12A.—Basal End-elevation of Dilated Type, Stage 2.

6. *Varicated Type*. Figs. 7 and 7A.

These, instead of having a faintly striated shell of even texture, have the body-whorl raised in a series of longitudinal ridges, which in some cases are very marked. Cf. Baker (1911), pl. xl, figs. 20 and 28.

7. *Dilated Type, Stage One.* Figs. 11 and 11A.

This type has probably developed from a varicated type where a single varication has increased very greatly to form a longitudinal swelling as illustrated.

8. *Dilated Type, Stage Two.* Figs. 12 and 12A.

A further development from the above, perhaps involving two enlarged varications.



FIG. 13.—Extreme Dilated Type.



FIG. 13A.—Basal End-elevation of Extreme Dilated Type.



FIG. 14.—Deflected-lipped Type, Stage 1.



FIG. 14A.—Basal End-elevation of Deflected-lipped Type, Stage 1.



FIG. 15.—Deflected-lipped Type, Stage 2.



FIG. 15A.—Basal End-elevation of Deflected-lipped Type, Stage 2.



FIG. 16.—Extreme Deflected-lipped Type.

9. *Extreme Dilated Type.* Figs. 13 and 13A.

An extreme development of the last. Baker illustrates nothing like any of these last three types.

10. *Deflected-lipped Type, Stage One.* Figs. 14 and 14A.

In this type the lip, instead of being perpendicular to the substratum, is deflected so as to lie parallel to it, forming a narrow rim. Cf. Baker (1911), pl. xl, fig. 21.

11. *Deflected-lipped Type, Stage Two.* Figs. 15 and 15A.

Here, the same variation has gone further, and the lip has "flared" out to form a flat expanse. Baker gives no figures of *S. catascopium* like this, but cf. his figures of *S. pallida*, Baker (1911), pl. xl, fig. 5; also that of *L. stagnalis* from Sea of Aral. Cf. Cooke (1895), p. 85, fig. 34c.

12. *Extreme Deflected-lipped Type.* Fig. 16.

This is the same variation carried to its extreme. No illustration of *S. catascopium* resembles it, but cf. figure of *R. peregra* from Sea of Aral. Cf. Cooke, *loc. cit.*, fig. 33b.

13. *Reflexed-lipped Type.* Figs. 17 and 17A.

This variation is another development from Type 10, where the lip, instead of remaining flat, has recurved upwards.



FIG. 17.—Reflexed-lipped Type.



FIG. 17A.—Basal End-elevation of Reflexed-lipped Type.



FIG. 18.—Doubly Reflexed-lipped Type.



FIG. 18A.—Basal End-elevation of Doubly Reflexed Type.



FIG. 19.—Twisted Type.



FIG. 20.—Ribbed Type.



FIG. 20A.—Basal End-elevation of Ribbed Type.

14. *Doubly-reflexed Type.* Figs. 18 and 18A.

This is the final development of the last, where the lip has recurved on itself, so as to form a white rim, which may be very heavy. Baker figures nothing resembling this or the last type.

15. *Ribbed Type.* Figs. 20 and 20A.

This type, which is rather less common, though by no means rare, is perhaps one of the most interesting of all. It shows a very strong longitudinal rib-like callosity, and may also be varicated. It would appear as though growth had been arrested at an earlier period and that a heavy lip of Types 13 and 14 had developed; then, favourable conditions returning, growth became normal again. Cf. Baker (1928), pl. xvii, fig. 29, and (1911),

pl. xl, fig. 22; and of *L. decollata* (1911), pl. xli, fig. 8. None of these figures, however, shows this variation so markedly.

16. *Twisted Type.* Fig. 19.

In this rather rarer type, the axis has been characteristically twisted in the manner illustrated, so that the aperture becomes rather reniform in outline. Cf. Baker (1911), pl. xl, fig. 2.

The relative dimensions of the shell show quite as much variation as the form of the shell itself. A few examples are noted below:

1. *Breadth of Shell to Length of Shell.*

Two shells each 13.2 mm. long. One is only 7 mm. broad, while the other is 11.4 mm. broad (including the expanded lip).

2. *Height of Aperture to Length of Shell.*

Two specimens 13.2 mm. long (actually the same two shells just mentioned). One has the aperture only 7.2 mm. high, while the other has it 10.6 mm. high.

3. *Width of Aperture to Breadth of Shell.*

Two specimens 8.9 mm. broad. One has the aperture only 5 mm. wide, while the other has the aperture 7.7 mm. wide.

4. *Width of Aperture to Height of Aperture.*

Two specimens. One has the aperture 9 mm. high, with a width of only 5 mm.; the other has an aperture 8.9 mm. high, with a width of as much as 8.4 mm.

5. *Breadth of Shell to Height of Aperture.*

Two specimens. One has the aperture 7.7 mm. high, with breadth of shell only 6 mm.; the other, with height of aperture 7.5 mm., has shell 8.9 mm. broad.

The reader is referred to the dot-graphs (6, 7, 8, 9, and 10) for the proportionate variation under the above headings.

(2) *Variation in Lip, Form, etc.*—An attempt was made to put in tabular form the incidence of the different variations in lip and form, etc., with a view to discovering the frequency of variation, and also the stage in development at which such variations became manifest.

Method: 1600 specimens of all sizes between 1.5 mm. and 16.5 mm. long were examined and classified for their various shell-characters, relative length and breadth, etc., *not* being taken into account. The categories were, of course, rather ill-defined owing to the great number of intermediate forms, but a moderately accurate survey was made.

Results and Conclusions: From the table it was seen that, out of 1600 shells, 1143 showed no variation in form from normal, but 480 of these were 5 mm. or less in size and were disregarded. Of the 1120 over

5 mm. in size, 59.3 per cent. (663) were normal, and 40.7 per cent. (457) showed variations.

28.8 per cent. (323) were varicated, 15.4 per cent. (172) being varicated *only*.

6.1	"	(68)	"	Type 7	3.0	"	(34)	being also varicated.
3.4	"	(38)	"	" 8	1.7	"	(19)	"
0.7	"	(8)	"	" 9	0.4	"	(4)	"
5.5	"	(61)	"	" 10	3.0	"	(34)	"
3.0	"	(33)	"	" 11	1.5	"	(17)	"
1.0	"	(11)	"	" 12	0.3	"	(3)	"
2.5	"	(29)	"	" 13	1.3	"	(15)	"
3.1	"	(35)	"	" 14	1.9	"	(22)	"
0.7	"	(8)	"	" 15	0.5	"	(6)	"
0.4	"	(5)	"	" 16	0.3	"	(3)	"

The writer can think of no reason why the incidence of Type 14 should be greater than that of Type 13 other than sampling error; nor can he find any genetic ratio in the figures.

From the table also the following percentages were calculated, and graphs made from the figures obtained (graphs 2 to 5) to show the incidence of variations with increase in length of shell. In the very small shells, discrepancies occur because of the difficulty of observing variations, while in the largest shells, inaccuracies are due to lack of numbers.

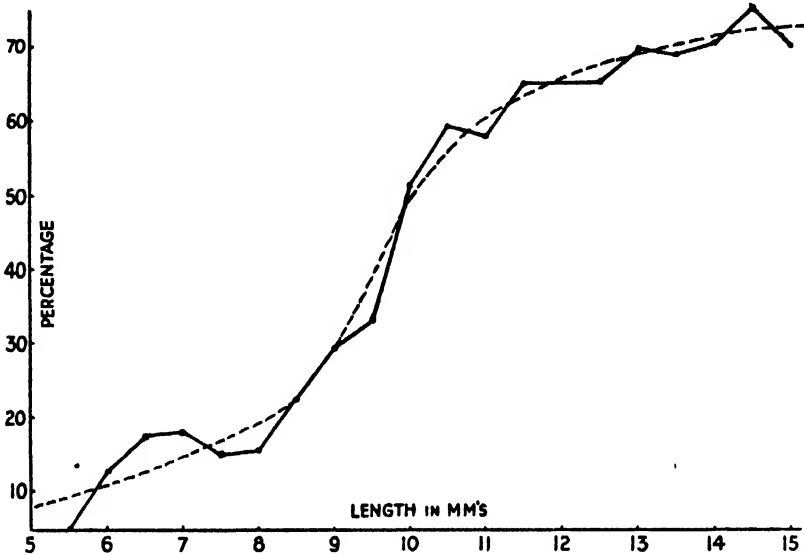
TABLE OF PERCENTAGES.

Length, mm.	Total Variants, per cent.	Including Variation, per cent.	Dilatation of Body-whorl, etc., per cent.	Variation of Lip, per cent.
5 and under
5½	4.9	4.9
6	12.9	10.3	1.3	1.3
6½	17.7	12.9	4.8 *	..
7	17.9	14.3	1.8	1.8
7½	15.1 †	15.1	..	1.9
8	15.6 †	12.5 †	1.6	3.1
8½	22.6	15.3	10.2 *	3.4
9	29.4	25.5 †	7.8	7.8
9½	33.3	24.5	8.8	8.8
10	51.4	29.7	9.5	25.7 †
10½	59.4	39.4	14.1	25.4
11	58.0 †	42.0	15.9	21.7 *
11½	65.1	40.3 †	12.5 †	36.1
12	75.0 *	45.8	16.7	43.7
12½	65.6	47.8	19.4	28.3
13	69.7	50.0	24.2	22.7
13½	69.0	52.4	30.9	19.0
14	70.4	59.2	25.9 *	36.9 *
14½	75.0 †	58.3	41.7	25.0 *
15 and over	70.0	30.0 *	50.0	10.0

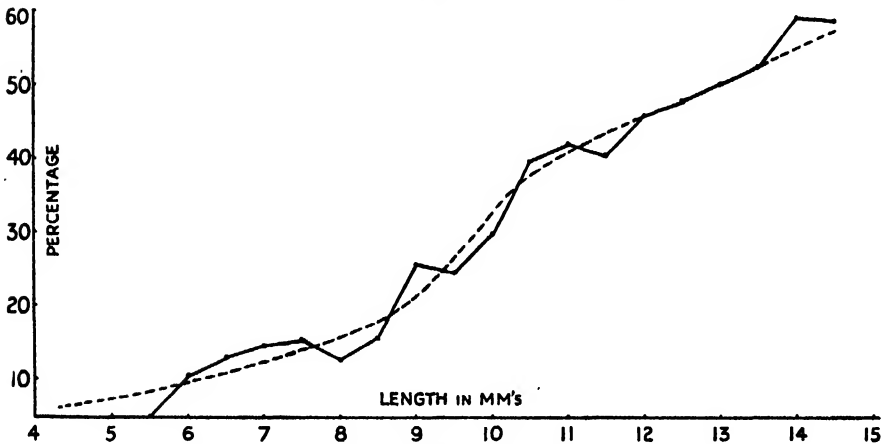
* Shows cases of sampling error marked ○ on graphs.

† Shows probable cases of sampling error.

Graph 2. Illustrates the general increase of all the variations in form taken together, compared with that of the size of the shell. It shows that the incidence of variation has its greatest increase between



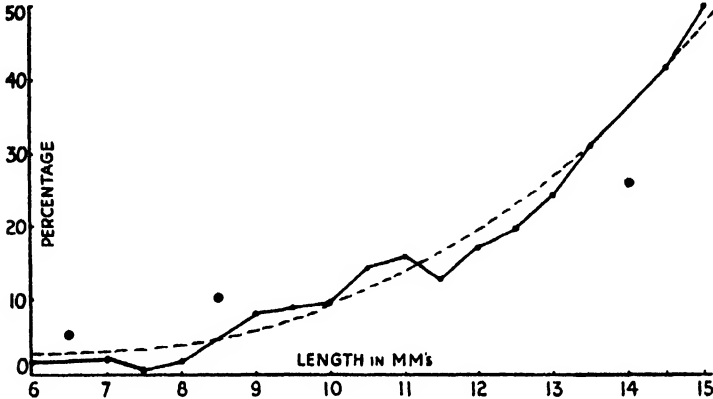
GRAPH 2.—Incidence of Total Variations.



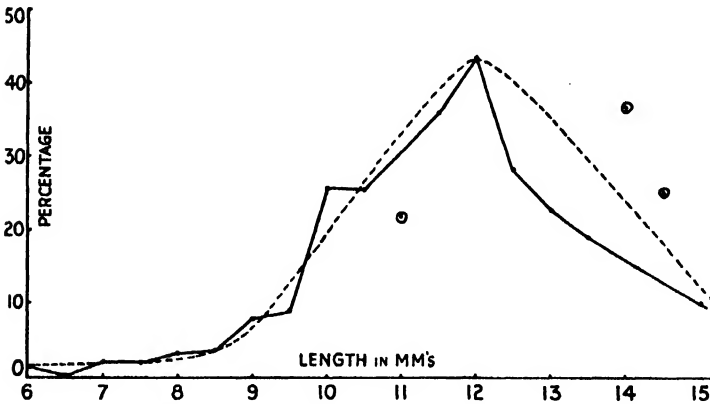
GRAPH 3.—Incidence of Variation.

8 mm. and 10.5 mm., after which the rate of increase is considerably lessened. In other words, if no variation is shown by the time 10½ mm. is reached, the shells are not so liable to vary, but this conclusion is a generalised one, and is not necessarily true for any one type of variation. Cf. graphs 3 and 4.

Graph 3. Illustrates the increase of incidence of varication with that of length. It shows much the same results as the last, excepting that the rise in the curve between 8 mm. and 10½ mm. is less sharp, while there is a much more definite increase after 10½ mm.—indicating a more



GRAPH 4.—Incidence of Variations in Body-whorl.



GRAPH 5.—Incidence of Lip Variations.

constant rate of increase of incidence. Therefore, shells frequently become varicated *after* they have reached this size.

Graph 4. Illustrates the increase of incidence of variations in the body-whorl, *i.e.* dilatation, etc. (Types 7, 8, 9, 15, and 16). It shows a gradually rising rate of increase of incidence corresponding to increase in size. Thus, the larger the shell, the more likely it is to have a dilated body-whorl.

Graph 5. This is the most interesting of the four graphs, and illustrates the increase of incidence of lip variations (Types 10–14). It shows

that there is but little occurrence of lip variation until after $8\frac{1}{2}$ mm. After this size, the rate rises sharply and fairly consistently till 12 mm. is reached, whereafter it decreases abruptly, becoming negative, so that the curve falls sharply down towards the larger sizes. Unfortunately the percentages at 14 and $14\frac{1}{2}$ mm. take away from the value of the graph, but even when these are given consideration there is still a rapid descent. From this it may be argued that specimens in which the lip has become greatly expanded or recurved cease to grow in length after about 12 mm., either dying more quickly than the others or, what is more probable, using all their shell-producing capacity for expanding the lip, which, having once begun to flare or recurve, would arrest spiral growth and any consequent increase in the length of shell.

(3) *Variation in Measurements.*—In addition to the very great variation of shell-form, there is also a very large variation in relative dimensions of the different parts of the shell, and some examples have already been cited. In an attempt to find some "order in chaos," the following measurements and calculations were made:—

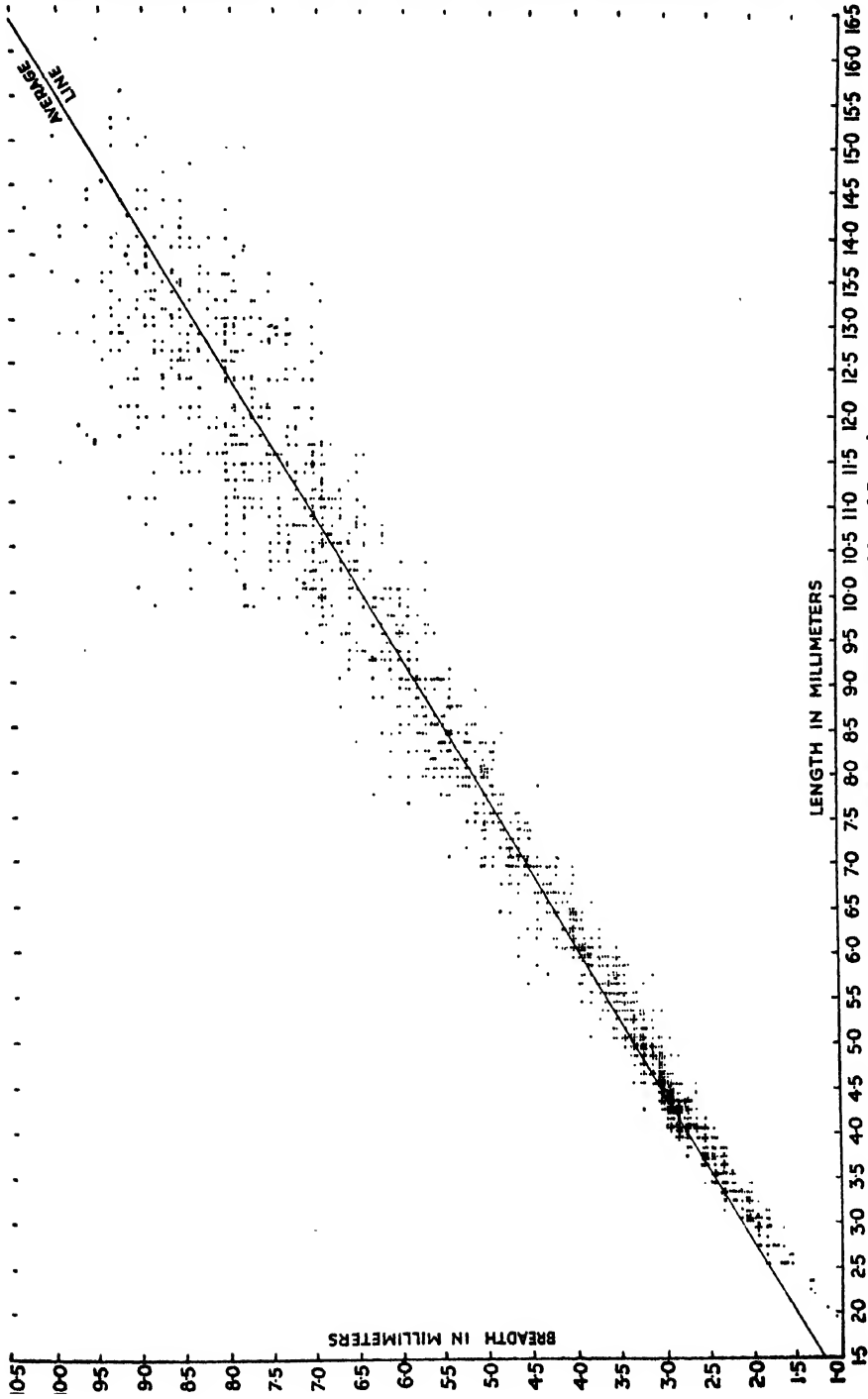
Method: 1600 shells were measured for length, breadth, height of aperture and width of aperture, to the nearest one-tenth millimetre, and five large-scale dot-graphs were prepared, Nos. 6, 7, 8, 9, and 10, comparing the frequency of variation of—

- (a) Breadth to length (6);
- (b) Height of aperture to length (7);
- (c) Width of aperture to height of aperture (8);
- (d) Width of aperture to breadth (9);
- (e) Breadth to height of aperture (10);

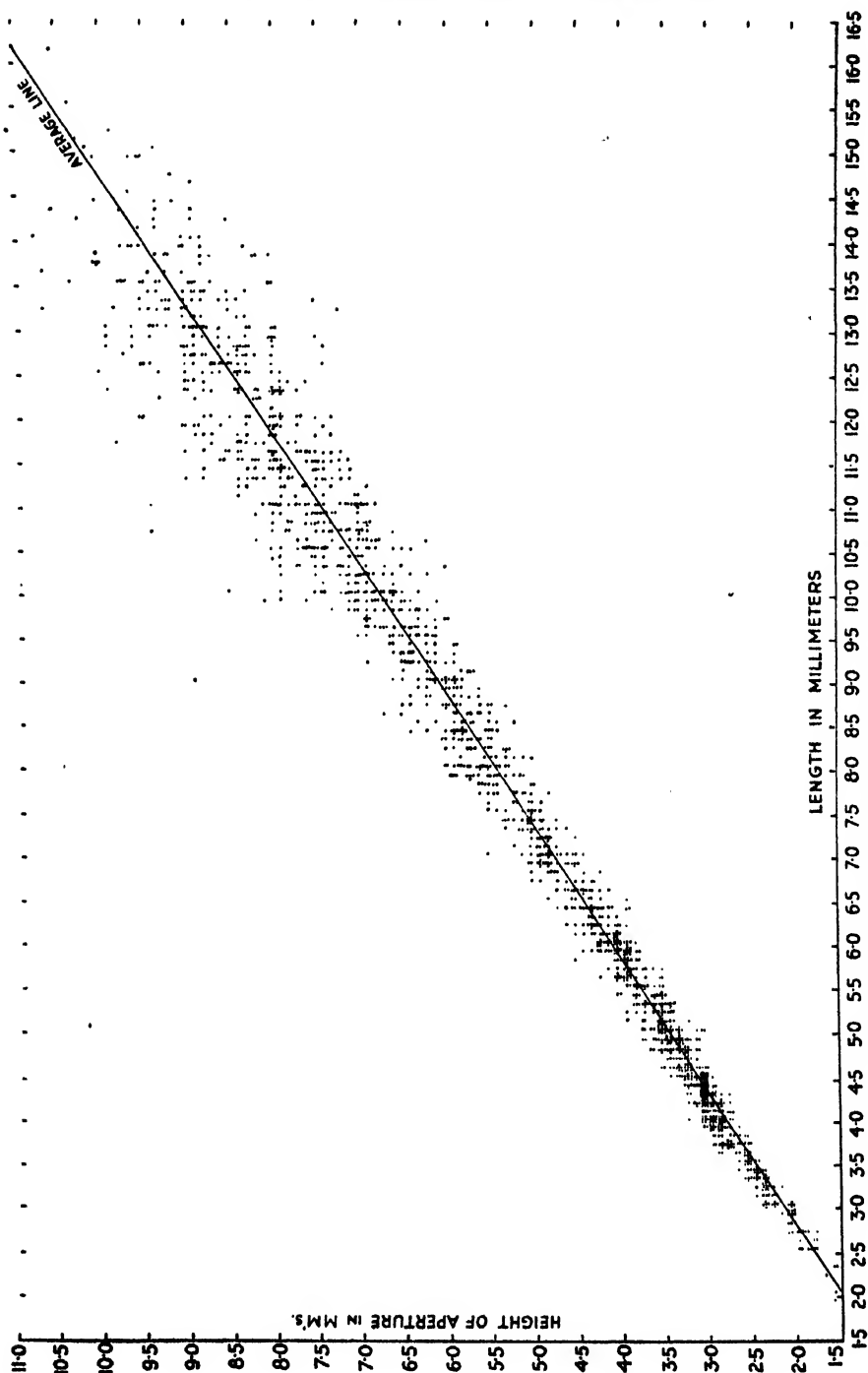
each square of the graph-paper representing $\frac{1}{10}$ th mm. in either direction, and each dot representing a single snail.

By calculations (see below) an average line—based on the "curve" of average increase in proportions—was added to each graph. These "curves" were found to be of the straight-line type, and were calculated in the following way:—

The abscissa of the graph in question was divided into columns, each representing 1 mm., and the number of dots in each column counted. The average dimensions of all the shells in each of these columns were calculated for each group, and the points plotted on graphs corresponding with graphs 6 to 10. Thus, in graph 6, there are 117 shells having a length between 8.0 and 8.9 mm. (inclusive) and varying in breadth between 4.8 and 6.5 mm. Their average length was 8.4 mm. and their average breadth 5.5 mm. This point was plotted on the corresponding graph.



GRAPH 6.—Frequency of Variation in Breadth and Length.



GRAPH 7.—Frequency of Variation in Height of Aperture and Length.

Below are the tables of these averages from which the curves of average increase in proportions were plotted.

BREADTH AND HEIGHT TO LENGTH.

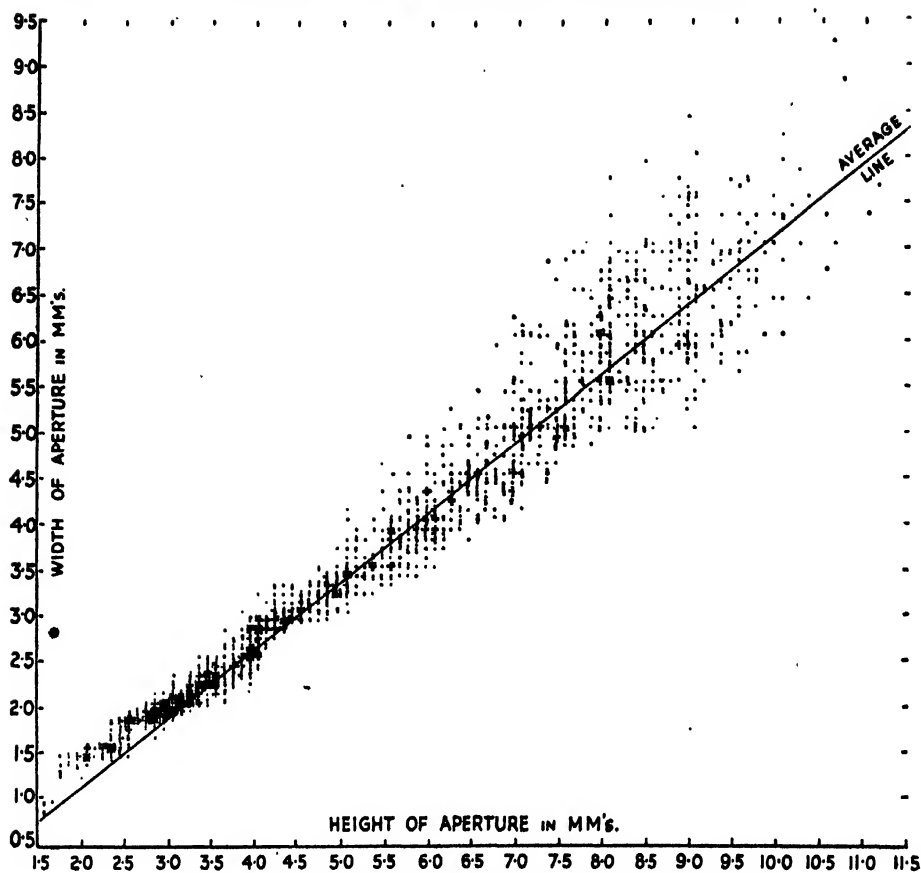
Graphs 6 and 7.

Number.	Length Group.	Average Length.	Average Breadth (6).	Average Height of Aperture (7).
	mm.	mm.	mm.	mm.
1	1.0 to 1.9
31	2.0 " 2.9	2.6	1.7	1.6
132	3.0 " 3.9	3.5	2.3	2.5
249	4.0 " 4.9	4.3	2.9	3.0
166	5.0 " 5.9	5.4	3.5	3.7
132	6.0 " 6.9	6.3	4.0	4.3
107	7.0 " 7.9	7.4	4.9	5.0
117	8.0 " 8.9	8.4	5.5	5.7
123	9.0 " 9.9	9.4	6.1	6.4
146	10.0 " 10.9	10.4	7.0	7.0
143	11.0 " 11.9	11.4	7.6	8.0
126	12.0 " 12.9	12.5	8.2	8.3
95	13.0 " 13.9	13.4	8.6	9.1
25	14.0 " 14.9	14.4	9.0	9.6
6	15.0 " 15.9
1	16.0 " 16.9
1600				

WIDTH OF APERTURE AND BREADTH TO HEIGHT OF APERTURE.

Graphs 8 and 9.

Number.	Height Group.	Average Height of Aperture.	Average Width of Aperture (8).	Average Breadth (9).
	mm.	mm.	mm.	mm.
20	1.0 to 1.9	1.7	1.2	1.6
205	2.0 " 2.9	2.6	1.7	2.4
340	3.0 " 3.9	3.4	2.2	3.2
185	4.0 " 4.9	4.4	3.0	4.2
176	5.0 " 5.9	5.5	3.8	5.3
174	6.0 " 6.9	6.5	4.4	6.2
198	7.0 " 7.9	7.4	5.3	7.2
196	8.0 " 8.9	8.4	6.1	8.2
87	9.0 " 9.9	9.3	6.6	8.7
17	10.0 " 10.9	10.2	7.4	9.4
2	11.0 " 11.9
1600				



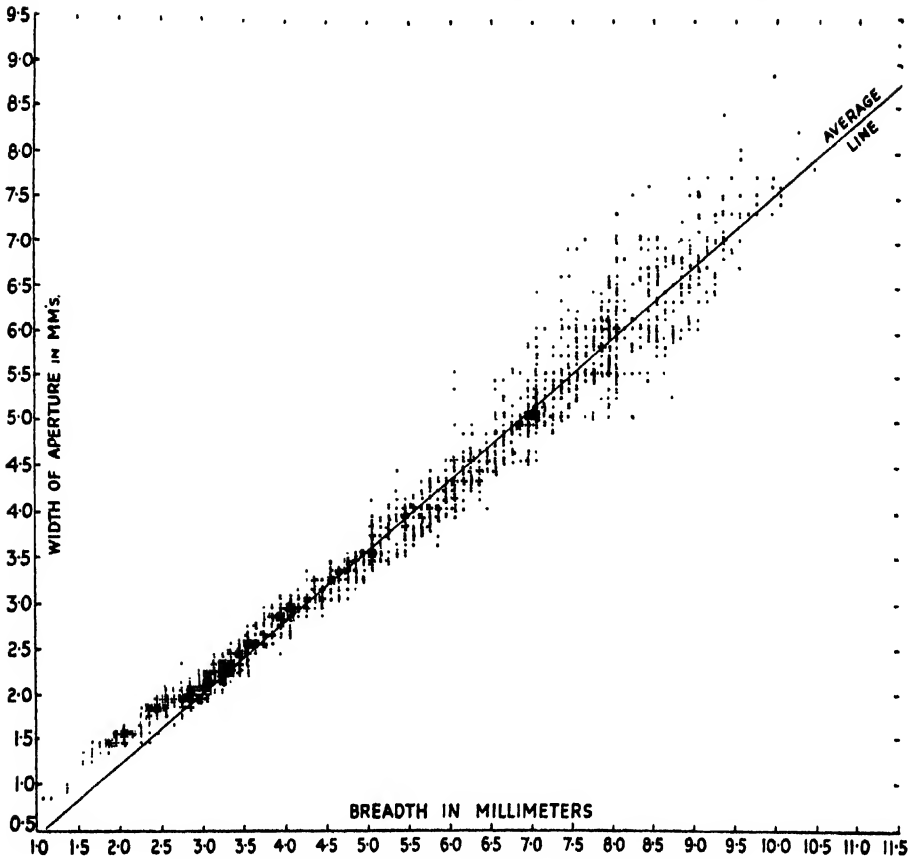
GRAPH 8.—Frequency of Variation in Width and Height of Aperture.

WIDTH OF APERTURE TO BREADTH.

Graph 10.

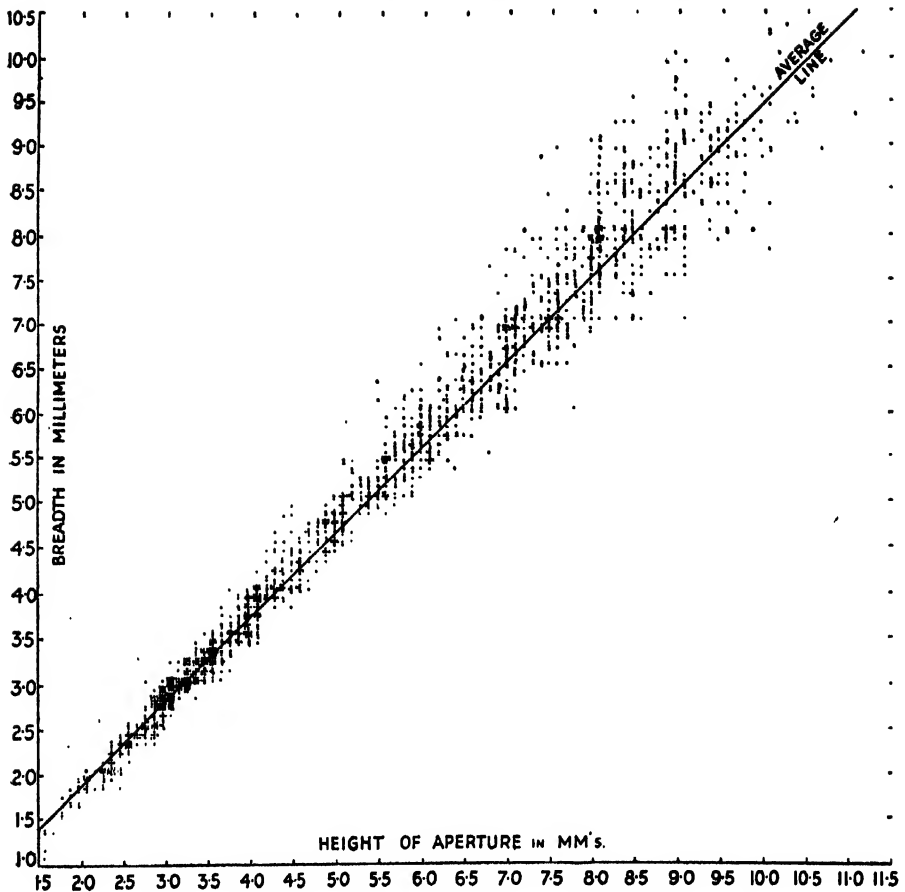
Number.	Breadth Group.	Average Breadth.	Average Width of Aperture (10).
	mm.	mm.	mm.
41	1.0 to 1.9	1.7	1.3
256	2.0 " 2.9	2.6	1.9
305	3.0 " 3.9	3.3	2.3
172	4.0 " 4.9	4.4	3.1
182	5.0 " 5.9	5.4	3.9
190	6.0 " 6.9	6.5	4.7
218	7.0 " 7.9	7.4	5.6
164	8.0 " 8.9	8.4	6.4
65	9.0 " 9.9	9.3	7.1
6	10.0 " 10.9
1	11.0 " 11.9

1600



GRAPH 9.—Frequency of Variation in Width of Aperture and Breadth.

Results and Conclusions: The dot-graphs 6 to 10 show that the phenomenal variation occurs in *all* measurements, and is not merely confined to, say, length and breadth. Naturally, if breadth varied, so would width of aperture, but this in turn would not necessarily vary in relation to the height of the aperture, as is clearly shown to be the case. Nor is this due to any great extent to the great variation of lip, as only 15 per cent. have such variation. Even the width of the aperture compared with the breadth of the shell, which one would expect to be more constant, varies greatly, though rather less so than the other proportions. The height of the aperture compared with the length of the shell one would also expect to be fairly constant, but this, too, is extremely variable. Further description of these graphs is unnecessary, and the reader is referred to the graphs themselves.



GRAPH 10.—Frequency of Variation in Breadth, and Height of Aperture.

From these graphs it may also be seen that the greatest variation occurs in the proportion of breadth to length (6); next, in width of aperture to height of aperture (8), followed by height of aperture to length (7) and breadth to height of aperture (9), with the proportions of width of aperture to breadth (10) showing least variation, though this, too, is very considerable.

Not only the large shells vary in shape, but also the small ones, which seem to vary almost as much in proportion to their size, as indicated by the fact that a line through all but the most extreme variations would be more or less straight, and not curved as would be the case if the shells began to vary after a certain size.

The tendency to vary in proportion, therefore, is fairly constant, and

would appear to be initiated at a very early stage—perhaps even before hatching—and the question of genetic inheritance should therefore not be overlooked. The whole question is extremely complex, as environment almost certainly has a very great influence, though what the actual environmental factor or factors may be has not yet been determined.

The result of the calculations showed that the “curves” of average increase in proportions were, in every case, straight lines, with extraordinarily little deviation, and it may therefore be noted that there would appear to be no general tendency to alteration of shape with increasing size in fairly normal shells. How far this is true of forms with lip and other variations is not clear.

The average proportions of the “normal” shell from the Leith habitat can be worked out at any given size. These proportions are:

Length.	Height of Aperture.	Breadth.	Width of Aperture.
10.0	6.6	6.3	5.0

V. VARIATION OF SHELL IN AMERICAN SPECIMENS.

In different localities the shell of *S. catascopium* may vary very greatly, as illustrated by Baker (1911, 1928), but few accounts of variation in individual *loci* are given. The following, however, may be cited:—

(a) Forms living on the stony shore of the “Wide Waters” of the Erie Canal show some interesting variations in the outer lip (Baker, 1911, p. 387). (b) In the Lower Genesee River, N.Y., where the water is more or less quiet along the shore, the shells are quite thin [similarly, the Leith form, being in still water, is also fairly thin], and exhibit a large amount of variation in comparative length of spire, the very short-spined forms showing a tendency to approach the *pinguis*-like aspect. The spire varies from one-half the height of the aperture to more than equal its length (Baker, 1911, pl. xl, figs. 6–10). [The spire of Leith specimens may be as small as one-third of the height of aperture.] (c) On the subject of shell variation of *S. catascopium*, Walker (1895, pp. 2–6) says: “. . . The bottom of the lake [Pine Lake] is composed almost wholly of marl, except where it has been covered by a thin coating of sand washed in from the shores, so that, in consequence, both plant and animal life exist under very unfavourable circumstances. The level of the lake seems to have been lowered by the canal. . . . In the numerous pools, which are left along the shore, the *Limnæa catascopium* Say is found in great abundance and almost infinite variety. It varies in shape from the slender form usually found in the Great Lakes to the globose form described as *L. pinguis* by Say. . . . The Pine Lake examples have usually

a very thick, solid, opaque shell, and a large proportion are more or less distorted, the most common effect of their unfavourable environment being apparently to induce a very abrupt and rapid expansion of the outer lip, which in most cases is accompanied by a heavy callus deposit all round the aperture." This description could well be applied to the Leith shells, except that these are not thick, solid, and opaque.

VI. POSSIBLE CAUSES OF VARIATION.

Baker (1928, p. 254) states that the shells from Prairie and Chetek Lakes are developing rather wide apertures, which, he says, "appears to be a direct response to a change from a river to a lake habitat . . . as the bodies of water were artificially made by damming the Chetek River, hence the original habitat for all lake forms was a river." Leith specimens, however, in still water, show all types, narrow- and wide-apertured specimens being equally common.

Walker (1895) gives no explanation for the occurrence of the distortion of shells other than "unfavourable circumstances." That "unfavourable circumstances" cause such distortion of the aperture, as is evident in the Leith specimens, is seen also in other species of *Lymnaea*, e.g. in *L. stagnalis* and *L. (Radix) peregra* from lakes near the Sea of Aral (cf. Cooke, 1895), which are salt for some months and comparatively fresh for others. What the actual adverse factor (if such exists) affecting shell-formation by the mantle may be has not yet been determined. Several possibilities present themselves:

(1) *Temperature*.—The most obviously abnormal condition in the Leith pond is the high temperature, and this at first would appear to be the most likely cause of the very wide variation. Against this conclusion, however, are the following facts: (a) The effect of excessive heat (or cold) is to produce thin dwarf forms (Cooke, 1895, p. 84), while Leith specimens are neither dwarfed nor exceptionally thin. (b) Similar variations occur under normal conditions (cf. this paper, Section V). (c) Other species of *Lymnaea* under thermal conditions show no development of these distortions, e.g. *L. (S.) palustris* taken from a hot spring (Yosemite) at a temperature of 80° to 90° F., show no abnormality, as illustrated by Brues (1927, p. 199, fig. 18); or *L. (Radix) auricularia* (Baker, 1911, p. 183 and pl. xxii), from a greenhouse (Chicago) at 90° F., regarding which no mention is made of abnormality, and the illustrations do not show differences in appearance from naturally occurring European specimens. Neither has the writer observed any distortion in *Lymnaea* from hot-houses in British

Botanic Gardens (e.g. Edinburgh, Belfast, Dublin). In addition, states Brues (1927), "several species of *Limnæa* live in thermal waters of Europe, Iceland and America, invading water of a rather higher temperature [than *Physa*] $\text{---}37^{\circ}$ to 45° C." These show no ill effects. If the temperature were to fluctuate, this might make the mantle secrete irregularly, causing varication, but in Leith there is no significant fluctuation, and in any case specimens bred experimentally at a constant temperature (27° C.) became varicated.

(2) *Pollution*.—The pond usually has a film of oil on at least part of its surface, which, presumably, comes from the condenser, and this pollution may tend to produce abnormality; but, on the other hand, Baker (1911, p. 387), referring to the pollution by sewage at Rochester, N.Y., says that, after seven years, the effect on the snails was a variation towards a *palustris*-like shell, which is in direct contrast to the type of variation found at Leith.

(3) *Calcium Content of Water*.—Distortion might be due to sub-normal calcium content. The pH of 7.5, however, indicates no great lack of calcium in solution, and almost certainly the annual lime-washing of the walls would provide ample lime for shell production—calcium in solution probably not being necessary if other sources are available (Boycott, 1936, p. 162).

(4) *Genetic Mutation*.—The possibility of genetic inheritance being involved with regard to measurements has already been indicated, but in the case of lip and body-whorl distortion, where counts were made, the writer found no significant ratios.

(5) *Other Possible Factors*.—Crowding might have some influence on the amount of food for each individual, and cause irregular growth, but by virtue of the high rate of juvenile mortality, a natural balance in economy would be attained. The food-supply itself might vary and bring about periods of activity, alternating with inactivity, causing the mantle to secrete irregularly and accounting for varication. Such an explanation might hold in a place where the environment might be liable to considerable variation from time to time, such as at Pine Lake (Walker, *loc. cit.*), but in Leith the environment remains constant all the year round, differing only in the amount of light. In addition, variation appears to be just as great among specimens taken when the food-supply of filamentous algæ was superabundant (1930). The possibility of parasitism of the snails causing the abnormalities must not be overlooked. Rothschild (1936), in a paper on *Peringia ulvæ* Penn., has noted variations caused by the cercariæ of Trematodes. The variations, however, were the production of exceptionally large

specimens, distorted spires, and the "ballooning" of the whorls. None of these variations occurs in the *S. catascopium* from Leith. No cercaria was found in the bodies of the snails, and, in any case, no definitive host would be likely to have access to them.

VII. EXPERIMENTS IN AQUARIA.

From the foregoing it will be seen that by mere observation practically no conclusion can be reached as to what is the actual cause of the great variation to which allusion has been made. Experiments were therefore begun under artificial conditions in the hope of finding an explanation. So far, however, these experiments have only been of a preliminary nature, and the number of snails involved was not sufficiently large to render counts significant.

(1) *Temperature*.—Egg-capsules were taken from the Leith pond at the end of July 1940 and hatched under different conditions of temperature. Hatching took place within three weeks of transference.

(a) *At 27° C. (constant)*. Resulting snails varied from the smooth type to the varicated and even ribbed form, some showing deflected lips, Stage 1. The majority were varicated only. Dilated lips did not occur—probably due to the small numbers involved. The relative proportions of snails from the same batch of eggs, however, seemed to be similar. A solitary ribbed specimen showed that this abnormality arose from an arrest in growth, followed by a slight deflection and then continued growth. The cause of the "resting period" is not known, but may be connected with nutrition, although conditions were the same as for those that showed no ribbing. These snails varied between 9 and 12 mm. in length in June 1941. (b) *At Room Temperature (averaging about 15° C.)*. In June 1941 these snails had only attained an average of 6 mm. in length, but, even at this small size, slight deflections of the lip occurred, though varications were not obvious and no other variations appeared. The relative proportions of the snails from this batch of eggs were again similar. (c) *At Outdoor Temperature*. The snails from eggs kept at outdoor temperature were even smaller than those of group (b), but varication had already appeared, although no other variation occurred.

Other evidence was provided by adult snails similarly transferred and allowed to continue growth under the same three conditions of temperature. The new growth was in most cases easily distinguishable, for, on being put into the aquaria, growth ceased for a while until the

snail had become adapted to the new environment. When this happened, a slight rim separated the old growth from the new. The general results at all three temperatures were similar: some snails continued to grow as before, while others developed abnormalities which had not previously been present, or such abnormalities as they already had became accentuated in certain cases. For example, one specimen (at outdoor temperature) showing a dilated lip, Stage 2, eventually showed this variation in its extreme form before it died, while the production of ribbed forms occurred even with an abundant food-supply. Dilated or deflected lips developed in "normal" types at all temperatures. In the case of the transferred adults it would appear that the adverse factor causing variation is the transference from one environmental condition to another, but in a few cases where there was a sufficient new growth it appeared that, even after adjustment had taken place, further abnormalities, such as the formation of a small secondary rib, arose. That distortion occurs at any temperature has been suggested, but so far not proved by breeding experiments, but the evidence provided by the adult snails kept under the three differing conditions of temperature shows that this is the case. Therefore temperature alone cannot be regarded as the cause of distortion. Other species of *Lymnaea* (*L. stagnalis*, *L. (R.) peregra*, and *L. (S.) palustris*) kept at 27° C. showed no distortion.

(2) *Pollution*.—Chemical pollutions all resulted in the death of the snails, while organic pollution (from an accumulation of putrefying vegetation) merely resulted in a black incrustation on the shells, the snails suffering no ill effects from their unsavoury environment. New growth in the snails observed showed no outstanding feature.

(3) *Calcium Deficiency*.—Certain adults were put in rain-water (at constant temperature) and the lack of calcium had the following effect: The subsequent growth was much thinner, with or without the various abnormalities, and erosion of the existing shell frequently took place, either by the action of other snails or by solution into the water after the periostracum had been damaged. Snails in the Leith pond show neither erosion nor shells so thin as in the new growth of experimental animals.

(4) *Genetic Mutants*.—With a view to discovering if there were any genetic factor involved, single snails, known not to have been cross-fertilised, were isolated. Unfortunately, only one laid eggs, and in the resulting snails there was a heavy mortality, the only result being that, from a "normal" type, there were produced, under the same environmental condition, a normal, a varicated, and a ribbed form.

This would tend to suggest that some genetic factor may be causing some variations, but until more is known about the genetics of self-fertilising snails it would be unwise to do more than mention the possibility.

(5) *Overcrowding*.—That crowding is not a factor causing abnormality was shown by the fact that specimens which were isolated varied just as much as those which were crowded together.

(6) *Nutrition*.—Variation was found to be just as great where there was an abundant food-supply as where food was scarce.

From the above preliminary investigations it would appear that neither high temperature nor any other environmental condition so far examined is a determining factor in inducing variation in the shells, although possibly influential. It would seem, therefore, that the variations may be explained on a genetic basis, although various difficulties arise in this connection.

First, there is the fact that certain snails, if transferred from one environment to another, may develop certain variations not previously shown; and secondly, that ribbing is caused by a cessation followed by a resumption of growth, the reason for which is often unaccountable, since it occurs in some specimens and not in others under identical and constant conditions even from the time of hatching.

Until further work is done, the only suggestion the writer can put forward is, that it is the *tendency* to vary which is inherited, and not any particular variation itself. This tendency to vary, or variation potential, is manifested under certain environmental conditions and not under others. Some variations, however, may be entirely environmental, and yet others entirely genetic—perhaps change in relative proportions or the occurrence of variation may be due to this latter cause. This hypothesis is only tentative, however, and is advanced in the absence of more tangible evidence. It may be that the tendency to vary is acquired (induced by the high temperature of the pond), and that if a sufficient number of generations were passed under natural conditions (of temperature) this tendency would be lost. If this could be proved experimentally it would go a long way towards solving the problem.

The following information with regard to life-history was also obtained from aquarium studies:—

(a) The length of life of individual snails kept at 27° C. is about one year. At lower temperature it is rather more. (b) The number of capsules laid by a single individual appears to be one. (c) Snails kept at 27° C. become adult in about nine months, though this is

probably longer than the period required in the Leith pond. Growth rate was greater in some specimens than others. At room temperature (about 15° C.) development is much slower, while at outdoor temperature even longer is required. Temperature therefore has a very marked influence on the *rate* of growth.

VIII. SUMMARY AND CONCLUSIONS.

Stagnicola catascopium (Say) is a common North American river and lake Lymnæid which has become naturalised in a warm engine-pond at 27° C. in the Scottish port of Leith, whither it has been transported on logs from Canada between 1900 and 1929, the pond being repopulated annually after cleaning by emergence of snails from the overflow pipes.

It is very prolific, though less so than formerly, averaging about 54 individuals per square foot of concrete, and showing a preference for ledges and the topmost foot of water. In the Leith habitat it feeds on non-cellular algæ (since the eradication of filamentous growth), and perhaps on Bryozoa.

The associated fauna is meagre (perhaps much reduced since the removal of the matted algal flora), a species of *Cyclops* and one of *Tubifex*, however, being very abundant. From the numerous statoblasts it would appear that *Plumatella repens* is also very frequent.

From 10 to 54 eggs are laid by *S. catascopium* at a time, and there are two main broods per year, though breeding is more or less continuous all the year round. There is a very high mortality in the second brood, presumably due to competition. Self-fertilisation can occur when the snails are isolated.

The high temperature of the pond causes a considerable increase in the rate of growth.

There is almost endless variety in the form of the shells in the Leith environment, apparently caused by some factor in this habitat affecting the secretion of shell by the snails.

There are about sixteen main types of shell with very many intermediates; the principal variations in form are (a) sudden expansion and/or reflexion of the lip, or (b) a modification of the body-whorl to a varicated or dilated appearance. Varication is by far the commonest variation, nearly 30 per cent. of all specimens examined showing this.

Incidence of varication increases fairly constantly with size, showing, however, greatest increase in shells of medium size. Dilatation of the body-whorl becomes more frequent as the shells become larger, while shells

showing lip variations do not increase much in length after they have reached about 12 mm.

Exceptional variations in the actual proportions of the shells also occur, which variations are initiated at an early stage, perhaps prior to hatching. The proportions of any individual shell appear to remain fairly constant throughout life.

Such variations in form and proportions are not confined to the Leith specimens of *S. catascopium*, but also occur in those found in America. This makes it difficult to determine the actual cause of their occurrence, especially as this may be quite different in America (*e.g.* Pine Lake) from what it is in Leith.

From the experimental evidence so far available, it would appear that environmental conditions, such as high temperature, pollution, lack of calcium, etc., play no determining rôle in the occurrence of variation (although possibly influential) and that this may have a genetic explanation involving the hypothesis of an inherited variation potential, which, however, might be acquired, and which, if a return to a normal environmental condition were made, might, after a number of generations, be lost.

Ribbing is apparently caused by a cessation in growth followed by a resting period before further development proceeds. The cause is probably environmental but difficult to determine.

The whole question of variation in *Stagnicola catascopium* is, under the conditions found in Leith, a highly complex one, and awaits further experimental investigation, as well as microscopic study of the mantle of abnormal forms.

IX. ACKNOWLEDGMENTS.

The writer's thanks are due to Professor James Ritchie for his help and encouragement; to the directors, staff, and employees of Messrs Park Dobson & Co., Ltd., Leith, for their co-operation and valuable information; and to Mr D. K. Kevan for his useful advice.

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XXXI.—Hair Pigmentation and the Genetics of Colour in Greyhounds. By Marca Burns, Zoology Department, University of Edinburgh. (*Communicated by Professor JAMES RITCHIE, M.A., D.Sc.*) (With One Plate.)

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CONTENTS.

	PAGE		PAGE
I. INTRODUCTION . . .	462	V. HAIR PIGMENTATION . . .	472
II. MATERIALS. . . .	463	1. Technique	473
III. ANALYSIS OF LITTER RECORDS . . .	463	2. Size and Form of Hairs. . . .	473
1. Relations of Black, Brindle, Fawn, Blue, and White-Spotted . . .	464	3. Pigmentation	475
2. Blue-Brindle and Blue-Fawn	469	VI. DISCUSSION. . . .	480
3. White-Flecked	471	VII. ACKNOWLEDGMENTS . . .	482
IV. COLOUR OF EYES, NOSE, SKIN, AND TOE-NAILS . . .	471	VIII. SUMMARY AND CONCLUSIONS . . .	483
		TABLES I TO V	484
		REFERENCES TO LITERATURE . . .	489
		DESCRIPTION OF PLATE . . .	490

I. INTRODUCTION.

THE present paper is the first step in an attempt to combine study of the genetics of coat colour in dogs with histological examination of the hairs and pigmentation, so that a picture may eventually be formed of the genetic relations of the various phenotypes, and of the processes by which the various colours are produced. Work along similar lines has been carried out by other workers on various mammals, *e.g.* on the mouse (Dry, 1926, 1928; Dunn, 1936; Einsele, 1937), guinea-pig (Wright and Hunt, 1918; Russel, 1939; Heidenthal, 1940), and in cattle (Bogart and Ibsen, 1938).

The number of published papers dealing with the genetics of coat colour in dogs is small, considering the material available, and only two papers deal with greyhounds. Dighton (1923) analysed records from the Greyhound *Stud Book* of the National Coursing Club (England) for the years 1907 to 1922, and determined the general relations of the main coat colours in greyhounds. His conclusions are, however, somewhat confused by failure to recognise white as the extreme form of white-

spotting, and by his treatment of the *Stud Book* records without allowing for the fact that litters registered in the *Stud Book* are not necessarily complete litters. The position is clarified by the work of Warren (1927), who used the American *Stud Book* records, and whose paper lays a sound basis for the understanding of colour inheritance in the breed.

Warren gives full descriptions of the four common colours which occur among greyhounds (black, brindle, fawn, and blue) as well as of white-spotting, and these have not therefore been described in detail here.

II. MATERIALS.

The first part of this work is based on the Litter Records kept by the National Coursing Club (England). These records are preferable to the *Stud Book* records in that they indicate the total number of puppies born, while only those which survived long enough to be registered are indicated in the published *Stud Book*. It has thus been possible to distinguish between depleted and undepleted litters, so that in calculation of ratios any error due to selective destruction of puppies by breeders can be eliminated.

A total of 1272 litters was registered during the twelve months (1937-38) covered by Vol. 58 of the *Stud Book*, and form the basis for the present analysis. These have been supplemented by a few litters registered in other volumes of the *Stud Book*, and by information obtained direct from breeders.

In addition to the work on litter records, greyhounds have been examined, their colours noted in detail, and where necessary a sample of the coat removed for microscopic examination.

III. ANALYSIS OF LITTER RECORDS.

Each litter was recorded separately on a card, as described by Warren, and the cards subsequently arranged according to the colours of the parents and offspring. In the table then drawn up reciprocal crosses were separated, but as this arrangement showed no indication of sex-linkage or other influence of sex on colour the separation has not been maintained in the tables presented here.

Of the 1272 litters registered in Vol. 58, 316 were depleted litters, *i.e.* one or more pups died or were destroyed before the litter was registered, and the colour of these pups is unknown. Depleted litters are indicated in the tables by the letter "d" following their number, and the same letter is used to indicate the number of dead puppies. Thus the entry "Litters 5 + 2d, Pups 35 + 5d" indicates that the seven litters, of which

two were depleted, contained 35 pups whose colours are known, plus five (dead) pups whose colours are not known. Of the 316 depleted litters, 29 had such a small proportion of survivors (generally only one or two) that they have not been included in the tables; 956 litters were not depleted. The colours of a total of 7719 puppies are recorded in Table I. The average number of pups per litter, including those which died, was 6.5.

Attention in this analysis has been concentrated on two aspects of colour inheritance: (a) confirmation of the relationship of the colours black, brindle (striped), fawn, blue, and white-spotted, described by Warren, and (b) the relations of three colours not previously described, namely, blue-brindle, blue-fawn, and white flecked.

1. *Relations of Black, Brindle, Fawn, Blue, and White-Spotted.*

Table I summarises, according to the various colours involved, the results of all matings registered in Vol. 58 of the *Stud Book* (excepting the 29 seriously depleted litters which were discarded).

As a result of his analysis of the American records Warren concludes that the colours black, brindle, and fawn (including red) in greyhounds depend on an allelomorphic series of extension factors E (producing black), E' (producing brindle), and e (producing red and fawn). E is dominant to E' and e, while E' is dominant to e. Blue colour is produced by a dilution factor, probably recessive, acting on E (black) and independent of the extension series. White-spotting probably depends on two main factors, one producing a predominantly white animal, and the second, which is dominant or epistatic to the first, producing a predominantly coloured animal with extensive white markings. Both factors for white-spotting are recessive or hypostatic to self colour, and the amount of white is affected by modifying factors. Self-coloured animals almost always have some white on the chest and toes.

These conclusions are in the main confirmed by the following analysis of the British records.

Relations of Black, Brindle, and Fawn.—Table II shows the results to be expected, if Warren's interpretation is correct, from the various matings between black, brindle, and fawn (including red) hounds; and shows also the actual results recorded in the Litter Records. Matings involving blues are not included in this table, but, in accordance with Warren's conclusion that blues are dilute blacks, blue offspring are included as blacks. The inheritance of white-spotting is discussed in a later section, and is not shown in Table II; the presence of white markings should not affect the relations of the coloured parts.

The method of analysis here adopted necessarily introduces some error, since litters from heterozygous parents may chance to contain no pups of the recessive colour (brindle or fawn as the case may be), and may therefore be included in the wrong group. Some relative excess of recessives is therefore to be expected among the offspring of all segregating matings.

The number of litters containing the recessive colours only gives some indication of the extent of error due to the wrong classification of litters containing only dominants, and is therefore noted in the last column of Table II. The litters referred to are in all cases included in the total in the preceding column.

Where the dominant concerned was black it was sometimes possible to check by other matings the genotype of the black parent or parents of litters containing only black offspring. In such litters from black \times black in only one case was one parent definitely homozygous EE (pups 6 + 3d); one litter (7 pups) was $Ee \times EF'$, two were from $Ee \times Ee$ (10 pups), and the remaining two (8 pups) could not be checked. In 12 litters from black \times brindle parents where all offspring were black, the black parent was homozygous EE in 9 cases (pups 42 + 7d), carried brindle EE' in one case (2 pups), and was of unknown genotype in the remaining two cases (12 pups). In 8 litters from black \times fawn where all offspring were black, the black parent was homozygous EE in 5 cases (pups 23 + 8d), heterozygous EE' in two cases (4 pups), and unknown genotype in one (4 pups).

In the circumstances a statistical test of agreement with Warren's hypothesis has not been made.

When due allowance has been made for blacks included in the wrong class there still remains a very considerable deficiency of blacks in Table II, class 2.c. The deficiency of brindles in relation to fawns in this group is not more than can be accounted for by their erroneous inclusion in class 2.b., but, even if the 14 pups in the 3 litters of blacks not known to have a homozygous black parent are added to class 2.c., this raises the blacks to only 194; and still leaves a deficiency of 86 blacks, of which less than a third could reasonably be attributed to erroneous inclusion in the previous class (2.b.). There appears therefore to be a real deficiency of blacks in this class, for which no explanation has been found. There appears to be a similar though smaller deficiency of blacks in class 3.b., though not in class 3.c.

In class 4.b. there is a deficiency of 192 brindles in relation to fawns, despite the fact that there is only one litter (of one pup) containing fawn and no brindle offspring. It would appear possible from this either

that lightly brindled hounds are frequently erroneously registered as fawns, or that the lowest degree of brindling may be phenotypically fawn. The latter possibility is supported by the widely varying degree of expression of the brindle character, which is suggestive of polygenic control of the amount of brindling; and also by the fact that a comparatively large number of matings of fawn \times fawn throw litters containing some brindle offspring, which litters are therefore classified as aberrant (Table I). However, if this is so, the deficiency of recessives (fawns) in class 5.b. is remarkable, especially when it is noted that 15 litters totalling 62 pups contained fawn pups only, indicating that a similar number of brindle pups may have been erroneously included in class 5.a.

It must therefore be concluded that while the records here examined do in general support the hypothesis of Warren that black, brindle, and red or fawn are due to allelomorphic genes, certain substantial exceptions remain unexplained. These may be due to a more complex genetic situation, or may be of no genetic significance, but attributable to unavoidable deficiencies in the material here presented, which render it unsuitable for full statistical analysis.

Fawn and Red.—The 82 undepleted litters from matings of fawn \times fawn were analysed for the three shades fawn, red-fawn, and red (Table III). In the *Stud Book* the letters "RF" officially stand for "red or fawn," since it is not always possible to say to which category a young puppy belongs; but breeders usually take it to mean "red-fawn" and may therefore register distinctly red animals as "RF." On the other hand, the number of distinctly fawn animals registered as "RF" is probably very small. There is no sharp distinction between red-fawn hounds and reds. No evidence has been found to support the view of Dighton (1923) that red in greyhounds corresponds to chocolate in other mammals.

Warren considers that fawn is probably recessive to red and due to a dilution factor which is not, however, the same factor as that which "dilutes" black to blue. The figures in Table III hardly support the view that fawn is recessive to the darker shades, even when allowance is made for the fact that some blue-fawns which are genetically red (see p. 480) are registered as fawn; this would account for the occurrence of a few reds as a result of matings of fawn \times fawn, but it would not account for the large number of litters from fawn \times red-fawn matings which contain fawn pups only, when not more than 50 per cent. of fawn offspring would be expected.

The possibility is not excluded that fawn may be due to a fourth allelomorph of the extension series. If this is the case, and if fawn is recessive to red and red-fawn, then matings of fawn with black and

brindle should give either fawn or red-fawns among the offspring, but not both. In order to test this possibility the matings of self-fawn \times self-black and self-brindle were separated from the corresponding matings of red-fawn (including red). Table IV gives the result of this analysis; black, brindle, and blue pups are not shown. Only 11 out of the 69 litters from matings of fawn \times black or brindle included both red-fawn and fawn puppies, and these exceptions might well be attributed to the recognised difficulty of distinguishing the red and fawn colours. But a very similar proportion of litters from red-fawn \times black or brindle matings contained both fawn and red-fawn offspring, whereas from these matings a large proportion of such litters would be expected; and even if red-fawn animals are all heterozygous for fawn (only the reds being homozygous red), litters containing fawn only should be the exception, but Table IV shows that of 103 litters from red-fawn \times black or brindle, 37 contained only fawn pups.

The whole question of the relations of red and fawn requires further investigation, and probably cannot be decided from records alone, mainly because of the difficulty of defining the colours involved with sufficient accuracy to avoid errors in registration.

The belief commonly held by breeders that fawn hounds throw blues more frequently than do other colours is not substantiated by the records either in matings of fawn \times fawn or, as seemed more possible, in matings of fawn with black or blue.

Blue.—Blue is not a common colour, and the number of matings involving blue is small. Only one mating of blue \times blue was registered in Vol. 58 of the *Stud Book*, and seven other volumes were therefore searched for such matings, and the total thus raised to six.

In calculating the ratios in Table II blue offspring have been included with blacks, on the assumption that they are black hounds which are homozygous for the recessive blue-dilution factor. It will be seen from Table I that matings which involve one blue parent support this assumption (*e.g.* the occurrence of blacks among the offspring of blues \times brindles or fawns), as does the spasmodic occurrence of blues among the offspring of black hounds. The symbol "dd" for blue-dilution can therefore be tentatively used. However, two of the blue \times blue matings threw blacks among their offspring. It would have been interesting to have examined the hair pigmentation of such blacks, assuming the records are correct, but unfortunately it has not been possible to follow them up.

One litter from blue \times fawn, and one from white-and-blue \times blue, included both brindle and fawn pups, while another remarkable litter comprised 7 brindle-and-white and 4 fawn pups with no black or blues—but it is possible that these litters were not correctly recorded.

Aberrant Litters.—There are 78 litters recorded in Vol. 58 which are obviously not in accord with Warren's findings, most of which contain puppies of colours considered by him to be dominant to those of the parents. These litters are listed separately in Table I. A number of similar cases occur in the data presented by Warren. Doubtless a proportion of such litters are due to errors in registration, or to deliberate misrepresentation, but it was considered that the matter should not be dismissed without further enquiry.

Of the 78 aberrant litters 16 are litters in which matings of black (or blue) \times black or blue or fawn give both fawn and brindle offspring, contrary to expectation. 31 litters are from matings of fawn \times fawn, which should throw only fawns, but these litters contain black, blue, and brindle puppies; there are 12 black or blue puppies and 71 brindles. Attention has already been drawn (p. 466) to the predominance of brindles among aberrant offspring of fawn \times fawn matings, which may indicate that some of the fawn parents are genotypically brindle. No explanation is offered of the other aberrant litters, but that some of these records are authentic is indicated by the two cases discussed below.

A number of breeders of known integrity were communicated with, and as a result of these enquiries it has been possible to verify one of the aberrant litters beyond all reasonable doubt. This litter was the result of a mating between a brindle dog (known from other matings to carry fawn) and a brindle-and-white bitch, and comprised one black-and-white bitch, two fawn-and-white bitches, and two fawn-and-white dogs. Despite the absence of brindle pups, the record seems to be authentic. The black-and-white bitch was the only black puppy born in the kennel that year, and there was no black or blue dog in the kennel at the time of the mating. The bitch in question is still alive, but has not yet been bred from. It is hoped that in due course it may be possible to determine whether she breeds as a black or as a brindle.

In a second case there is again no possibility of confusion between the aberrant litter and any other, since there was no other litter of the same age in the kennel; the possibility is perhaps not quite excluded that the bitch might have mated with another dog very early in the heat period, before she was recognised to be in season. Such a possibility is rather remote, and since the litter was born 67 days after mating with the registered sire of her litter it seems somewhat unlikely that the litter was really the result of an earlier mating. The bitch in question was black-and-white, while the sire was fawn. The 4 puppies were one black, two brindle, and *one red-fawn*. This red-fawn dog "Ted" is referred to later in discussing the pigmentation of red and fawn hairs, and is

considered by his breeder to differ in type from the other members of the litter.

I am greatly indebted to the breeders of these litters for their help and information.

White-Spotting.—The genetics of white-spotting is not exhaustively examined here, but it is evident that the records of the National Coursing Club confirm the conclusions of Warren concerning this character.

Table V includes only data from matings of blacks with blacks, blues, and brindles, except in the case of predominantly white \times predominantly white matings, of which only three occur in the whole of Vol. 58. These data agree well with Warren's finding that white spotted is recessive to self colour, and the greater amount of white recessive to predominantly coloured. The so-called "sels" thrown by matings between spotted hounds may be assumed to be merely an extreme form of spotting, as suggested by Warren, since almost all hounds registered as "self" do in fact have some white on them. White hounds, as Warren states, are merely the extreme form of "predominantly white," and breed as spotted hounds (Tables I, V, and VII).

It appears that white-spotting occurs considerably less frequently among the offspring of self-fawns than among the offspring of other self-colours. Thus the total undepleted litters from self-fawn \times self-fawn included 386 self, 58 predominantly coloured, and 20 predominantly white pups; while the corresponding total from matings of black \times black, and brindle \times brindle, self hounds includes 814 self, 369 predominantly coloured, and 40 predominantly white offspring. White markings are somewhat less conspicuous on a fawn animal than on the darker shades, and this possibly leads to animals being registered as self-fawn despite white markings which, in a darker animal, would lead the breeder to register it as spotted. However, the matter merits further enquiry.

2. *Blue-Brindle and Blue-Fawn.*

While Warren mentions the great variation in shade found in both brindle and fawn hounds, and distinguishes between fawns and reds, no mention is made of the two colours known to breeders in this country as blue-brindle and blue-fawn. In both these colours the coat has a "smoky" appearance, but otherwise the hound appears to be a typical brindle (with any degree of brindling), or else a fawn (but not red).

In the *Stud Book* a number of hounds are registered as blue-brindle or blue-fawn, but no doubt in many cases such hounds are registered simply as brindles or fawns respectively.

The idea suggests itself that these dogs are homozygous for the blue-

dilution factor, the genotype Edd being blue, E'dd being blue-brindle, and eedd being blue-fawn. In an attempt to test this three volumes of the *Stud Book* were searched for crosses between blue-brindle and blue, or reciprocally, but only three such matings were found. Of these the first is recorded as giving one brindle and one blue dog, and one brindle bitch. It was possible to trace the bitch, which is not phenotypically blue-brindle. The second mating gave black, blue, brindle, and fawn offspring, and each of these colours with white! If this record is correct the blue-brindle parent must have been heterozygous for blue-dilution, and both parents heterozygous for fawn and white-spotting. The third mating gave blue, blue-brindle and fawn offspring, and therefore conforms to the suggestion that blue-brindles are homozygous for blue-dilution. The evidence is not sufficient for any decision, but it is clear that all the blue-brindles did carry blue either in the homozygous or heterozygous condition. Blue-brindles also occur occasionally among the offspring of black or brindle hounds.

Concerning blue-fawn also, evidence from the *Stud Book* is unsatisfactory. In Vol. 58 none of the 59 fawn puppies from matings of blue \times fawn are described as blue-fawn; there are 28 fawns and 32 red-fawns (but some of those described as fawn may, of course, be blue-fawn). One mating between blue-fawn and fawn is recorded: among the 7 offspring 1 is described as blue-fawn, 3 as fawn, and 3 as red. From another mating, of fawn \times fawn, 6 blue-fawn puppies and 2 fawns comprise the litter of 8. In a litter from fawn \times red-fawn-and-white 1 blue-fawn-and-white pup occurs in the total of 6, the remaining 5 being red-fawn. In Vol. 54 two litters by the same blue-fawn dog are recorded. His mates are a fawn-and-white bitch and a red-fawn bitch, but both litters are registered as containing blue offspring, 5 blue-and-whites in one litter and 1 in the other.

From all matings where one parent is blue only one litter contains blue-fawn pups. This is a litter of 6 from a mating of blue \times brindle, which gave 1 blue-and-white, 1 red-fawn-and-white, and 4 blue-fawn-and-white puppies.

Apart from the *Stud Book* records, one of the blue-brindle animals examined undoubtedly carries blue-dilution and is very probably homozygous for this factor. She is registered simply as "brindle," but is a blue-fawn-brindle bitch ("fawn-brindle" is a term sometimes used by breeders to describe a very lightly marked brindle, in which most of the body is fawn, with perhaps only one small brindled area). Her dam was a blue, while the sire, a light fawn, threw many blues among his offspring. This bitch, like the other blue-brindles examined, has the light eyes and

greyish nose typical of blues. She will be referred to again in discussing the hair pigment (p. 479). One of her litter mates was blue.

The pigmentation of blue-fawn and blue-brindle hairs is discussed in a later section.

3. *White-Flecked.*

A colour pattern which has not been described in other breeds of dog occurs in greyhounds, and is known to breeders as "white ticked." In order to avoid confusion with the coloured dominant ticking of hounds (Whitney, 1928) and with similar coloured ticks sometimes found on white-spotted greyhounds, this pattern will be referred to as "white-flecked." The pattern consists of numerous very small white flecks (groups of white hairs) in the coat of an otherwise self-coloured hound, or on the coloured areas of white-spotted hounds. It varies very much in degree of development, some white-flecked hounds having only perhaps a dozen white flecks in their coat, while in others the white flecks are very profuse; but a few white flecks are often present on coloured hounds, and are of no apparent significance.

The special interest of this pattern is that it develops gradually, often not appearing until the hound is several months old, or even over a year old; and the number of white spots continues to increase with age.

Owing to its late development white-flecked is not generally recorded in the *Stud Book* registrations of hounds, although a few, generally blacks in which the white flecks are more conspicuous than in other colours, are registered as "ticked." Unfortunately white-spotted hounds with coloured "ticks" are registered in the same way, so that the meaning of "ticked" in the *Stud Book* is not consistent. The evidence from *Stud Book* records of this pattern is therefore very incomplete, and information obtained from greyhound breeders has not made clear the genetics of "white-flecked"; indeed it may prove not to be a genetic character, although breeders regard it as heritable, and it does seem to "run in families." Flecked hounds appear spasmodically in the pedigrees, and seldom throw ticked offspring, but generally several of the litter-mates of a flecked hound are flecked. The variation in time of appearance as well as in the number of white flecks which may appear on a hound, seems to indicate that more than one factor is involved.

IV. COLOUR OF EYES, NOSE, SKIN, AND TOE-NAILS.

The eye-colour in greyhounds is often described by breeders as one of three shades, "dark brown," "medium," and "hazel" or "light," but there is some overlapping between the shades. The eyes of blue hounds,

which though light yellow show a certain "smokiness," are sometimes described as "grey." The darkest eye-colour seen was definitely brown, not black; the lightest was a pale amber shade.

In greyhounds, as in cocker spaniels (Barrows and Phillips, 1915), there is apparently no correlation between eye-colour and the colour of the coat, except in the case of blue hounds. A black hound may have very light eyes, and a pale fawn may have eyes of the darkest shade. Medium brown eyes are the most common, and light eyes are rather unusual except in blues. Definite evidence has not been obtained, but it seems probable that (non-blue) light eye-colour is recessive to the darker shades.

Of the eleven blue hounds whose eye-colour was examined, eight had the light smoky-yellow eyes which are regarded as characteristic of blues, but the remaining three had "medium" eyes. The eyes of these latter, when compared with those of non-blue medium-eyed hounds, are definitely "smoky" and not clear brown, and these animals possibly correspond to dark-eyed non-blue hounds. All the blue-brindle and blue-fawn hounds examined (about fifteen) had light smoky eyes except one very dark blue-brindle whose eyes were medium in colour.

Nose colour is black in all hounds, including white hounds, except in blues, blue-brindle, and blue-fawns, in which it is always greyish, even when the eyes are medium instead of light. Brown noses such as occur in some red cocker spaniels (Barrows and Phillips, 1915) were never seen in greyhounds, although one red had a rather brownish nose.

Colour of the skin on the body was not specially examined, although it was noticed where the hair is sparse, *e.g.* on the belly, that the skin may be pink or greyish, sometimes light with darker patches. In a red-fawn bitch the bare skin on the scar of a wound was intense black. In all hounds, including the palest fawns, and blues, the lips and rims of the eyes appear black, unless in a white-spotted hound they are included in a white area, when the portion in that area appears pink.

The colour of the foot-pads is greyish in all hounds.

Colour of the toe-nails appears to be quite irregular: the same hound may have dark, medium, and light toe-nails even on the same foot, and a white toe may have a dark nail, and conversely. Really black nails have not been seen on any blue hound.

V. HAIR PIGMENTATION.

It has not been possible to study the pigment of the coat colour as thoroughly as was originally intended, but as this work has to be temporarily suspended, and as the results of the examination of whole hairs

proved to be of considerable interest, some preliminary observations are given below. No reference to detailed examination of the hair pigment of the dog has been found in the literature, but Lochte (1938) gives a general description of dog hairs, with special reference to hairs of the German shepherd dog (Alsatian) and of the Dachshund.

1. *Technique.*

Most of the samples of hair collected consisted of combings from the shoulder region of the hound, but some samples included hairs from other parts. No attempt was made to distinguish hairs from different regions, except where there was some obvious colour difference on the dog. One sample was clipped; all other samples were naturally shed hairs and included the roots. Most of the samples were taken in September, but some were taken in the spring. The spring samples consist mainly of wool hairs probably indicating that overhairs are not shed so freely at that time of year.

Each sample was placed in a small envelope and stored in darkness. During subsequent preparation for examination unnecessary exposure to light was at all times avoided.

When required for examination the sample of hair was washed in absolute alcohol and ether-alcohol. Some were then rinsed in ether and dried, to be cleared and mounted later, or used for chemical tests as required. Other samples were transferred directly from the ether-alcohol to absolute alcohol for dehydration, and then taken through a series of alcohol-xylol mixtures to xylol, the process taking at least three days (Dry, 1926). After twelve hours in xylol the xylol was changed, and the hair, immersed in xylol, was exposed in a Hearson vacuum incubator for a few minutes, in a partially successful attempt to remove air-bubbles from the medulla. The hairs remained in the xylol for a further twelve hours or more, and were then mounted in thin Canada balsam. In taking the hairs up through the xylol mixtures each mixture was run under the previous one so that the hairs were not at any time exposed to the air. By this method fairly successful mounts were obtained, although the medullary spaces of the large overhairs almost always still contained gas bubbles, and many of the smaller hairs were not entirely free of them.

2. *Size and Form of Hairs.*

As the hairs were mounted whole, several on a slide, and without special orientation, the preparations were not suitable for detailed examination of structure, and no measurements of twist or of cross-sectional shape were possible.

Overhairs vary in length in different regions of the skin, the longest being those on the under side of the tail, while on muzzle and on feet the hair is very short. Hairs from the shoulder region are usually from 1.5 to 2.0 centimetres in length, and this is also the approximate length of the wool hairs in this region, although some wool hairs are considerably shorter, and longer ones also occur.

The overhairs are strong hairs with a backward curve but no crimp. They have a broad medulla which tapers off, becomes fragmentary, and finally disappears, both distally and proximally, so that at both ends of the hair there is a short region without medulla, or with only a few medullary fragments. In the large overhairs the medulla is of the compound, flattened type (Hausman, 1920). The air-spaces are relatively large, and the divisions between them thin. The cortex is fairly thick. An average overhair of length about 2 cm. has a width of from 0.05 to 0.1 mm. in its broadest part, and the medulla occupies about half the total width. The hairs taper towards the tip and also narrow in the basal non-medullated portion.

The undercoat fibres are very much finer than the overhairs, and are crimped. There are two main types, one with a regular simple ovate medulla, and the other in which the medulla is fragmentary and absent in parts. There are intermediate types which show various degrees of reduction of the medulla. In an undercoat fibre of the medullated type and about 2 cm. in length the medulla occupies about one-quarter of the total width, which is approximately 0.025 mm. These figures have no very exact significance because of the wide variation in size and type of the wool hairs. The number of crimps is usually from three to five. Some hairs, probably corresponding to the auchenes of Dry (1926), have a simple medulla, but either are not crimped or have two or three crimps in their proximal part and a straight and broader region at the tip.

Although the wool hairs are as long as, or often longer than, the overhairs of the same region, they are completely concealed by the overhairs and the coat has a smooth glossy appearance; the wool hairs form a thick matted protective undercoat.

The roots of overhairs are bulbous, those of wool hairs hardly broader than the shaft.

The scale structure has not been studied in detail, although one preparation from an overhair was made by the method described by Manby (1933). This, and observations on white hairs, have shown that the scales are arranged in the common imbricate fashion, and are ovate in the wool hairs, but tend to be more flattened and crenated in the large overhairs.

3. *Pigmentation.*

Pigmentation has been examined in hairs from hounds of the following colours: black, red, red-fawn, fawn, red-brindle, light brindle, blue, blue-brindle, blue-fawn-brindle, white. Brown ("rusty") hairs from a black hound were also examined. The hairs were mounted for microscopic examination as already described.

Durham (1904) found that the yellow melanin extracted from hairs of guinea-pigs was soluble in cold dilute alkali, whereas sepia pigment was not. The immersion for 48 hours of whole hairs, whether from black or red greyhounds, in potassium hydroxide solution, had no apparent effect on the pigment, even when the solution used was sufficiently strong partially to destroy the structure of the hair. Immersion in 10 per cent. acetic acid solution also had no apparent effect on the pigment. The bleaching effect of hydrogen peroxide solution on certain hairs is described below.

Black Hairs (Pl. I, figs. 1, 4, 7, 10).—The pigment of black hairs consists of sepia granules which are present in both medulla and cortex of both overhairs and wool hairs. In the cortex of the overhairs the granules are mostly arranged in short longitudinal rows which become more densely packed towards the tip of the hair. Irregularly scattered granules are also present. The cortex has a general reddish appearance which seems to be due to the many layers of granules and not to diffuse pigment, since in the basal region where granulation is sparse the reddish colour appears only around each row of granules. In all the untreated black overhairs examined the medulla appeared solid black throughout due to the presence of gas-bubbles, and no structure could be seen; however, in hairs treated with dilute acid (see below), which had no apparent effect on the pigment, the medulla was partially cleared and could be seen to contain numerous sepia granules, closely but rather regularly packed, with occasional small intense clumps of granules.

Black wool hairs show varying amounts of pigmentation. The average size of the sepia granules appears to be larger than in the overhairs, and when a medulla is present the pigment is concentrated between the air-spaces. Granules are present in the cortex, a greater proportion being scattered than in rows. Pigmentation is more intense distally than towards the base. The general impression from both overhairs and wool hairs is that the granules are very regularly and evenly distributed, in marked contrast to the condition in blue hairs described below.

Black overhairs were bleached by placing them in commercial hydrogen peroxide solution (10 volumes per cent.). They remained in this solution

for 27 days, the solution being changed occasionally. After the first 24 hours the black hairs had become distinctly lighter, "sepia" instead of "intense black." After 6 days the hairs appeared deep yellow or red in colour, with no trace of their original brownish-sepia tone. No further sharp change had taken place after 4 weeks, when they were removed from the hydrogen peroxide, washed, dehydrated, cleared, and mounted in the ordinary way. The hairs in this preparation appear yellow in colour like a red hair, although in the darkest region (sub-apical) the colour is darker than that in any red hair examined; nevertheless it is definitely red, not sepia, in tone. The granules are less evenly distributed than in a red hair, a greater proportion being in lines and fewer scattered. Otherwise the bleached black hair is indistinguishable from a dark red hair. As in red hairs there are a few small clumps of yellow pigment here and there. In the least coloured parts of the cortex it is not possible to determine whether the pale yellow pigment is diffuse or granular.

Brown ("Rusty") Hair of Black Hound.—The pigmentation of these hairs is similar to that of black hairs but less intense (compare black hairs from brindle hounds). "Rusting" is probably due to bleaching of some of the pigment by the sun, and, since some hounds "rust" more readily than others, is probably partially dependent on hereditary factors, as in "blackish" cattle (Bogart and Ibsen, 1938) and sheep (Roberts, 1927). "Rusting" tends to increase with age of the hound, and in the summer coat. Blue hounds also rust.

Red Hairs (Pl. I, figs. 2, 5, 8).—It appears to be characteristic of both red and fawn hairs that the basal half of the overhair, and generally of the wool hair, is colourless. Pigment is sometimes present in the medulla throughout its whole length, or the basal part of the medulla may be without pigment. The quantity of pigment in the medulla gradually increases distally, and a yellow tinge (probably small granules) commences in the cortex close to the medulla. The margin of the cortex appears colourless in this region, but this may be due to the smaller thickness at the margin. Nearer the tip the yellow pigmentation becomes intense in both medulla and cortex, and the granules can be well seen. They are distinctly smaller than those in black hairs and appear a clear reddish-yellow with no brown tinge. As in black hairs, some of the granules in the cortex are scattered, others are in longitudinal rows which are thinner than those of the black hairs because of the smaller size of the yellow granules, and are therefore less conspicuous. The coloured portion of the hair presents a general yellowish appearance, but careful examination indicates that this is due to small granules and not to diffuse

pigment. In the medulla of overhairs the granules are rather larger than in the cortex, and although plentiful in the coloured regions are not crowded together.

The arrangement of the pigment in red wool hairs is similar to that in the overhairs, but the wool hairs are paler, and the structural variation increases the variation in colour.

In both overhairs and wool hairs a few small dense clumps of yellow granules are visible.

On bleaching red hairs in hydrogen peroxide, as described for black hairs, they become distinctly paler in colour but remained definitely yellow even after a month in the solution.

Fawn Hairs.—There is no sharp distinction between the hairs from a pale fawn hound and those from a red, but there is a general reduction in the quantity of pigment, so that in many wool hairs there is practically none. Although some quite large granules occur, and an individual hair may be quite heavily pigmented, in general the individual pigment granules are very small and in the paler hairs it is impossible to be certain from a whole mount whether diffuse pigment is present or not. Sectioning, or the centrifuging method (Einsele, 1937) would be necessary in order to determine this point.

Red-Fawn Hairs.—The coat of the ordinary red-fawn hounds is similar to that of reds but lighter, and requires no special discussion. A sample of hair from the aberrant red-fawn dog "Ted" referred to on p. 468 will be described here. The hound in question is remarkable not only in that he is an unexpected fawn from a mating which should have thrown only blacks and brindles, but also in that his colour is peculiar. His general appearance is red, but numerous black hairs are interspersed with the red hairs all over the body, except on the face and paws. Many red-fawn hounds have black on the ears and tail. The clear red on the face and paws is rather reminiscent of the bicolour ("black-and-tan") pattern in other breeds, but the colour of many red and fawn hounds is lighter on the face than on the body.

This coat sample was clipped, so contained no roots. The red hairs which compose most of the sample appear quite normal in pigmentation. The black hairs, or certainly the majority of them, are black only in their distal part, and have a fawn band at the base. This band under the microscope appears yellow. In some black hairs the pigment granules appear to be sparsely scattered large sepia granules even in the fawn basal region, but in other hairs the pigment in the yellow band consists of very small yellow granules indistinguishable from the pigment found in red and fawn hairs. The yellow colour is confined to the medial layers

of the cortex as in the basal parts of most red overhairs, but distally when the pigmentation becomes denser and more brown, it extends towards the surface of the hair.

Red-Brindle and Light-Brindle.—The coat samples from both red-brindle and light-brindle hounds were collected in the spring and contained few overhairs. Both samples were found to consist of two quite distinct types of hair, those which resemble hairs from a black hound, and those which resemble the hairs from reds and fawns. Most of the black hairs are not so intensely pigmented as those from a black hound, and in their more sparsely pigmented parts have a yellowish appearance under the microscope, but with a slightly more brown tint than is found in red or fawn hairs, probably due to the absence of very small granules. The red and fawn hairs are quite typical and might have been taken from red or fawn hounds. Both overhairs and wool hairs show the two alternative types of colouring. No banded hairs were seen. The yellow wool hairs from the light brindle are almost colourless, with only a little yellow pigment in the distal end of the medulla; all distinctly coloured hairs from the light brindle have sepia pigment. The animal thus seems to correspond to a brindled fawn, while the red-brindle might be a brindled red. The genetic relations of the two types of brindle are not known.

Blue Hairs (Pl. I, figs. 3, 6).—The pigmentation of blue hairs differs sharply from that of black hairs owing to the presence of large, irregular, and very intense black, aggregations of pigment, which are most distinctive. These aggregations, in which no structure can be seen in the unbleached hair, are scattered through the cortex and medulla, with no regular arrangement. In addition to these black aggregations there are sepia granules in rows and scattered, but they are not quite so numerous as in black hairs. The total amount of pigment appears to be about the same in blue and in black hairs. Pigment aggregations are present in most of the wool hairs in addition to sepia granules. In almost all the overhairs and large wool hairs several aggregations of pigment lie medially in the non-medullated basal part of the hair, and this line of pigment aggregations continues into the root of the hair, which in black and other non-blue hairs is always free, or almost free, of pigment. In blue hairs the medulla is usually deformed, and in the large overhairs it is generally absent, except in a short region near the base.

On bleaching blue hairs in hydrogen peroxide, in the manner already described, the free granules became yellow as did those of black hairs, while the black aggregations became brownish-red and granular in appearance, closely resembling in colour the much smaller clumps of

granules occasionally seen in unbleached red hairs, and like the yellow aggregations in blue-fawn hairs (see below).

Blue-Brindle Hairs.—Only four overhairs from this hound have been examined. Two closely resemble overhairs from blue hounds, having sepia granules and intense black aggregations extending into the roots, but the medulla is not broken or deformed in either hair. The third overhair contains yellow pigment in both medulla and cortex in its proximal half, and distally the pigmentation consists of typical sepia granules and black aggregations as in a blue hair. The fourth overhair contains no aggregations and the basal two-thirds is entirely without pigment. A yellowish tinge precedes the definitely red region at the tip of the hair, in which granules can be seen. It is the only hair seen in which there is quite an extensive pigmented area in which no granules can be seen. The hair is described for this reason although there is a suspicion that the sample may have been contaminated during collection, and this hair may not really belong to the blue-brindle hound. The wool hairs from the blue-brindle are pigmented, some with sepia and some with yellow granules, as in ordinary brindle hounds. A few aggregations of pigment are present in most of the yellow wool hairs, especially towards the base, and they are more frequent in the sepia hairs.

Blue-Fawn-Brindle Hairs (Pl. I, fig. 9).—The hairs from this bitch are perhaps the most interesting of all the coat samples. They are of two types as in brindle animals. The most numerous have only yellow (red) pigment; there are the usual small free granules as in a normal red hair, but in addition there are typical aggregations of red pigment, with a granular appearance identical with the aggregations of a bleached blue hair. The second type of hair, which presumably corresponds to the black hairs of a normal brindle, is most remarkable in that it contains both black and red pigment aggregations. This is the only type of hair in which black and red pigment can be distinguished in the same region of one hair. The free granules cannot be divided into black and red types, but around each black aggregation a few large granules can be seen, which appear sepia, not yellow in colour. The wool hairs in this sample show little pigment in the cortex but yellow pigment is present in the medulla, and as two or three aggregations towards the base, but not in the root. In some of the longer wool hairs the pigment has a more sepia tint. No pigment aggregations were seen in the root of any hair from the blue-fawn-brindle, although they extend to the roots in both blue and blue-brindle hounds.

From the quantity of pigment present in the fawn hairs of this bitch it seems probable that they are blue-dilute red hairs, not fawn hairs.

It is likely that all animals registered as blue-fawn are red hounds with blue-dilution, since the quantity of pigment in ordinary fawn hairs is so small that the presence of blue-dilution could hardly have much visible effect, and a blue-dilute fawn hound is probably very hard to distinguish from an ordinary pale fawn hound.

White Hairs (Pl. I, fig. 11).—White hairs were collected from a brindle-and-white hound. These hairs, unlike the white hairs of spotted cattle (Bogart and Ibsen, 1938), contain no pigment whatever.

VI. DISCUSSION.

The microscopic examination of whole mounts of greyhound hairs has proved to be a valuable supplement to work on the *Stud Book* records, and tends to confirm the genetic findings based on the latter. For example, Warren's statement that fawn is not due to the same dilution factor as blue, is strongly supported by examination of the pigment. Certain problems which arose from the analysis of the *Stud Book* records can probably only be solved by a combination of direct observation and information from breeders, with further microscopical work: such problems include the genetic relation of red, red-fawn and fawn, and the undoubtedly complicated relations of the various shades of brindle.

The pigment in all greyhound hairs is probably granular; parts of the hair without granules do not show any colour. Only in one hair was there definite colour without visible granules, although in some very pale yellow hairs granulation could not be certainly seen throughout.

There appear to be two kinds of pigment granules—"sepia" and "yellow," but it is not clear whether the difference is qualitative, or whether the smaller size of the yellow granules accounts for the difference in colour. Nor is it clear whether the range of granule size in black hairs overlaps, or even includes, that in red hairs. The presence of both sepia and yellow pigment together in the blue-fawn-brindle hairs seems to indicate that there is some qualitative difference, and the bleaching of sepia pigment to red by hydrogen peroxide might be taken to indicate that red is a more highly oxidised melanin derivable from sepia. On the other hand this effect could be due to a simple reduction of the quantity of pigment, perhaps accompanied by a reduction in granule size, by the hydrogen peroxide; although if the hydrogen peroxide destroys the pigment (as does potassium permanganate, see Russel, 1939) one would expect small granules to be destroyed more readily than large ones. The effect on black hairs might be a simple unmasking of the yellow pigment by the destruction of sepia, but the effect of bleaching blue hairs, whereby

the black aggregations of pigment become red and visibly granular, seems to indicate that the sepia pigment is actually changed to yellow. Moreover, neither in black hairs nor in the similar but more lightly pigmented hairs from brindles, nor in blues, are any yellow granules visible in the unbleached hair, even where the sepia granules are most sparsely scattered; yet after bleaching both blue and black hairs show numerous scattered yellow granules, and it seems that these granules must be derived from the sepia granules of the unbleached hair. They are not, of course, necessarily the same as the yellow granules of red and fawn hairs despite their similar appearance.

The yellow granules of red hairs are certainly not of the same nature as the yellow pigments of guinea-pigs (Wright and Hunt, 1918) since in hounds the yellow pigment is not soluble in cold dilute alkali, and in this it resembles the sepia pigment of guinea-pigs.

Warren's interpretation of the genetic facts, summarised in his statement that "all colours in greyhounds appear to be due to extension factors acting upon black," may be applied to the distribution of pigment as follows: (1) The extension series (black, brindle, red, and fawn) controls the quantity of pigment and perhaps the size of the granules, in such a way that the base of the hair loses colour before the tip, and the cortex before the medulla; but the pigment is regularly and evenly distributed in that portion of the hair which is pigmented. In brindle hounds some follicles produce black hairs and others red (or fawn) with no intermediate types. Skin and eye colour are not affected by the extension series. (2) Blue-dilution produces clumping of some of the pigment granules into large irregular aggregations, which appear both in the cortex and in the medulla, and may be black or red in colour. Free scattered granules are also present. Castle (1931) states that in rodents the blue-dilution factor reduces the number of pigment granules and clumps them, but the distinctive arrangement of the pigment in blue greyhound hairs is very different from that found in a blue-dilute mouse examined. In the latter the pigment granules, which were all much larger than any seen in hounds, were concentrated in the medulla with only a few scattered in the cortex; but there was nothing comparable to the dense aggregations of pigment which distinguish blue-dilute greyhound hairs. Blue-dilute can affect black, brindle, and red hounds, and affects skin and eye colour as well as the colour of the coat. It is not clear from the records that blue-brindle and blue-fawn hounds are homozygous for the blue-dilution factor, but this is probable. (3) The white-spotting genes inhibit pigmentation, not within the individual hairs, but over certain areas of the coat. They affect the colour of the lips and rims of the eyes, but apparently

do not prevent black pigmentation of the nose (see illustrations of white-spotted hounds in Warren's paper).

The effects of the extension series of genes might be explained on the assumption that they affect the time of production of pigment relative to the growth of the hair, and the amount of pigment. Black hairs would be the result of a plentiful supply of pigment throughout the growth of the hair, while red and fawn hairs have a small supply of pigment available only in the earlier stages of their growth, so that they become colourless towards the root. This hypothesis does not, however, explain the growth of new hairs of the same colour after each moult (dogs normally lose some hair at all seasons of the year, but there is a definite moult generally in the early autumn, and often another in the spring; moult is much affected by health and feeding; it is not known what proportion of the total number of hairs is replaced annually). Hounds do tend to become rather lighter in colour with age (*e.g.* rusting in blacks), but the process is indefinite and very gradual, and the lighter appearance is partly due to greying (growth of white hairs) rather than to the growth of less pigmented hairs.

Many attractive problems remain to be solved. Quantitative study of sectioned normal and bleached hairs, and much more detailed genetic and histological examination of the various shades of brindled hound, are obviously required. The occasional occurrence of puppies whose colour does not conform to genetic expectation requires further investigation, as does the apparent shortage of white-spotted red and fawn hounds.

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VIII. SUMMARY AND CONCLUSIONS.

1. Evidence from 1272 matings recorded in the Litter Records of the National Coursing Club (England) tends to confirm the conclusions of Warren (1927) that the colours black, brindle, and red or fawn in greyhounds depend on allelomorphic genes; although certain substantial exceptions remain unexplained. The occasional occurrence of a pup of a colour dominant to its parents is noted, and it is suggested that some phenotypically red or fawn hounds may be genotypically brindle. It is suggested that fawn may be a fourth member of the extension series and not the result of a dilution factor acting on red.

2. The same records confirm Warren's conclusions in regard to the inheritance of blue-dilution and white-spotting.

3. Two colours, blue-brindle and blue-fawn, which are probably the result of blue-dilution acting on brindle and red respectively, are described and their genetic relations discussed.

4. The colour pattern "white-flecked" is described and discussed.

5. Colour of eyes, nose, skin, and toe-nails are described. Eye-colour is independent of coat-colour except in blue-dilute hounds.

6. Pigmentation of the hairs from hounds of various colours was examined microscopically in whole mounts. In all coloured hounds granular pigment is present, and diffuse pigment is probably absent. The apparent difference between the two types of pigment seen (sepia and yellow) may be due to different granule size, and not to any chemical difference. Hairs of blue, blue-brindle, and blue-fawn-brindle hounds all show distinctive aggregations of pigment (clumps of granules) in contrast to the even distribution of pigment in non-blue hairs. White hairs contain no pigment.

7. Black, red, and blue hairs were all bleached by hydrogen peroxide solution. The sepia pigment of black and blue hairs became red (deep yellow) in colour. Red hairs merely showed a lightening of the yellow pigment. Neither sepia nor yellow pigment dissolved in cold dilute acid or alkali.

8. If there is in fact only one pigment, sepia, in greyhound hairs, then Warren's statement that the various colours are due to extension factors acting on black can be applied literally to the pigmentation of the hairs. It is suggested that the extension series of genes (E black, E' brindle, e red or fawn) may take effect by controlling the amount of pigment available to the growing hair.

TABLES I TO V.

Note.—In the tables which follow, the following abbreviations, used in the National Coursing Club records, are sometimes used:—

Bk, black; Bd, brindle; R, red; RF, red-fawn; F, fawn; Be, blue; W, white. Where an animal is of mixed colour that predominating is written first, *e.g.* BkW, black-and-white; WBk, white-and-black.

TABLE I.—SUMMARY OF LITTER RECORDS, ARRANGED ACCORDING TO THE COLOURS INVOLVED.

CROSS: BLACK × BLACK.

Parents.	Litters.	Pups.	Bk	BkW	WBk	Be	BeW	WBe	Bd	BdW	WBd	F	FW	WF	W
(a) <i>Throwing Blacks and Blues only.</i>															
Self	4	19	11	6	..	1	1								
Spot.	1 + 2d	16 + 5d	6	10											
Total	5 + 2d	35 + 5d	17	16	..	1	1								
(b) <i>Throwing Blacks and Brindles (3 : 1).</i>															
Self	7 + 2d	67 + 2d	34	11	..	3	14	5					
Spot.	8 + 4d	79 + 7d	2	48	3	2	2	..	7	14	1				
Total	15 + 6d	146 + 9d	36	59	3	5	2	..	21	19	1				
(includes	15	108	30	42	3	5	14	13	1)				
(c) <i>Throwing Blacks and Fawns (3 : 1).</i>															
Self	none														
Spot.	7 + 1d	53 + 2d	15	20	1	2	1	9	2	3	
(d) <i>Aberrant Litter.</i>															
Self	1	7	..	3	1	..	1	2			
CROSS: BLACK × BLUE.															
(a) <i>Throwing Blacks and Blues only.</i>															
Total	4	28	3	19	6								
(b) <i>Throwing Blacks and Brindles (1 : 1).</i>															
Total	4 + 1d	22 + 6d	8	4	3	7					
(d) <i>Aberrant Litter.</i>															
BkW × Be	1	10	..	2	1	..	4	1	2	
CROSS: BLACK × BRINDLE.															
(a) <i>Throwing Blacks and Blues only.</i>															
Self	7 + 2d	51 + 5d	37	4	3	7									
Spot.	3 + 2d	26 + 5d	10	12	1	..	1								
Total	10 + 4d	77 + 10d	47	16	4	7	1								
(b) <i>Throwing Brindles and Blacks (1 : 1).</i>															
Self	36	197	42	33	4	7	3	1	73	31	3				
Spot.	41	258	43	69	8	8	6	2	56	58	6	2
Total	77	455	85	102	12	15	9	3	129	89	9	2

Hair Pigmentation and the Genetics of Colour in Greyhounds 485

Parents.	Litters.	Pups.	Bk	BkW	WBk	Be	BeW	WBe	Bd	BdW	WBd	F	FW	WF	W
<i>Depleted Litters (b).</i>															
Self	12d	62+25d	7	16	..	1	2	..	24	12					
Spot.	9d	46+27d	5	10	2	..	1	..	15	9	4				
Total	21d	108+52d	12	26	2	1	3	..	39	21	4				
<i>(c) Throwing Blacks, Brindles, and Fawns (2:1:1).</i>															
Self	33	219	50	30	1	10	3	..	32	15	3	61	13	1	
Spot.	32	224	23	45	9	1	8	..	33	25	15	28	25	12	
Total	65	443	73	75	10	11	11	..	65	40	18	89	38	13	
<i>Depleted Litters (c).</i>															
Self	10d	67+15d	26	6	..	4	..	1	10	1	..	17	2		
Spot.	7d	43+16d	3	11	1	2	3	10	2	1	8	2	
Total	17d	110+31d	29	17	1	4	..	3	13	11	2	18	10	2	
CROSS: BLACK × FAWN.															
<i>(a) Throwing Blacks and Blues only.</i>															
Total	5+5d	43+12d	26	14	..	3									
<i>(b) Throwing Blacks and Brindles (1:1).</i>															
Self	15	97	27	11	2	33	21	3				
Spot.	16	120	13	35	1	..	7	..	20	44					
Total	31	217	40	46	3	..	7	..	53	65	3				
<i>Depleted Litters (b).</i>															
Self	9d	56+17d	22	9	19	6					
Spot.	8d	57+13d	15	13	20	8	1				
Total	17d	113+30d	37	22	39	14	1				
<i>(c) Throwing Blacks and Fawns (1:1).</i>															
Self	37	245	49	42	1	9	10	2	102	28	2	
Spot.	19	128	15	48	3	3	3	36	17	2	
Total	56	373	64	90	4	12	13	2	138	45	4	
<i>Depleted Litters (c).</i>															
Self	3d	18+4d	3	3	6	6		
Spot.	5d	32+14d	..	10	2	..	3	3	14		
Total	8d	50+18d	3	13	2	..	3	9	20		
<i>(d) Aberrant Litters.</i>															
Self	3+1d	35+1d	2	8	3	7	7	1	6	1		
Spot.	7+1d	59+1d	3	19	1	14	..	9	13		
Total	10+2d	94+2d	5	27	3	8	21	1	15	14		
CROSS: BLUE × BLUE.															
Total	6	36	2	2	..	9	4	4	7	1	1	3	3		
CROSS: BLUE × BRINDLE.															
Self	18+2d	119+4d	23	12	..	16	13	1	29	8	1	7	9		
Spot.	8+4d	78+12d	9	17	4	6	3	..	6	16	3	5	9		
Total	26+6d	197+16d	32	29	4	22	16	1	35	24	4	12	18		
CROSS: BLUE × FAWN.															
Self	10+7d	105+14d	24	21	..	8	1	1	7	3	..	35	5		
Spot.	5+4d	55+8d	6	8	3	4	6	..	3	4	1	7	11	2	
Total	15+11d	160+22d	30	29	3	12	7	1	10	7	1	42	16	2	

Parents.	Litters.	Pups.	Bk	BkW	WBk	Be	BeW	WBe	Bd	BdW	WBd	F	FW	WF	W
<i>Blue x Fawn Aberrant Litters.</i>															
Be x F	1	9	..	1	1	2	5		
Be x WF	1	11	7	4		
CROSS: BRINDLE x BRINDLE.															
<i>(a) Throwing Brindles only.</i>															
Self	41	257	180	71	5	1
Spot.	37	224	101	102	21	
Total	78	481	281	173	26	1
<i>Depleted Litters (a).</i>															
Self	12d	60 + 28d	22	34	4	
Spot.	13d	67 + 30d	38	19	6	4
Total	25d	127 + 58d	60	53	10	4
<i>(b) Throwing Brindles and Fawns (3 : 1).</i>															
Self	68	458	201	108	9	92	41	7	
Spot.	48	292	82	96	17	36	49	10	2
Total	116	750	283	204	26	128	90	17	2
<i>Depleted Litters (b).</i>															
Self	26d	164 + 71d	71	38	3	34	16	2	
Spot.	13d	78 + 26d	13	25	9	9	20	2	
Total	39d	242 + 97d	84	63	12	43	36	4	
<i>(d) Aberrant Litters.</i>															
Total	10 + 2d	83 + 3d	9	12	4	1	2	1	28	9	8	2	6	..	1
CROSS: BRINDLE x WHITE.															
Bd x W	1	8	1	1	3	1	..	1	
<i>(This White breeds as a Fawn.)</i>															
CROSS: BRINDLE x FAWN.															
<i>(a) Throwing Brindles only.</i>															
Self	25	161	124	36	1	
Spot.	16	88	32	53	3	
Total	41	249	156	89	4	
<i>Depleted Litters (a).</i>															
Self	9d	46 + 15d	27	17	1	1
Spot.	7d	32 + 20d	5	20	4	3
Total	16d	78 + 35d	32	37	5	4
<i>(b) Throwing Brindles and Fawns (1 : 1).</i>															
Self	135	880	310	120	16	325	96	9	4
Spot.	88	596	122	159	30	108	139	34	4
Total	223	1476	432	279	46	433	235	43	8
<i>Depleted Litters (b).</i>															
Self	42d	258 + 101d	98	29	4	89	37	1	
Spot.	21d	121 + 33d	22	29	3	36	27	3	1
Total	63d	379 + 134d	120	58	7	125	64	4	1
<i>(d) Aberrant Litters.</i>															
Self	7 + 2d	63 + 5d	8	9	..	4	1	..	32	3	..	5	1		
Spot.	6 + 4d	78 + 6d	3	19	1	2	..	1	11	16	7	8	8	2	
Total	13 + 6d	141 + 11d	11	28	1	6	1	1	43	19	7	13	9	2	

Parents.	Litters.	Pups.	Bk	BkW	WBk	Be	BeW	WBe	Bd	BdW	WBd	F	FW	WF	W
CROSS: FAWN × FAWN.															
(a) <i>Throwing Fawns only.</i>															
Self	54	341	299	41	1	
Spot.	28	196	50	107	38	
Total	82	537	349	148	39	
<i>Depleted Litters (a).</i>															
Self	22d	106+49d	87	17	2	
Spot.	8d	41+18d	16	21	4	
Total	30d	147+67d	103	38	6	
(d) <i>Aberrant Litters.</i>															
Self	11	74	1	6	21	1	27	17	1	
Selfd	8d	38+16d	1	1	1	..	14	5	1	10	2	2	
Spot.	10+ 2d	80+ 4d	1	4	3	..	5	14	4	12	24	10	
Total	21+10d	192+20d	1	4	1	2	4	..	25	40	6	49	43	13	
CROSS: FAWN × WHITE.															
W × FW	1d	7+2d	..	2	1	2	2	
W × WF	1	7	1	4	1	..	1	
(Both these Whites breed as Blacks.)															

TABLE II.—OFFSPRING EXPECTED FROM VARIOUS COLOUR CROSSES.

The ratios expected theoretically, and those given by the litter records are shown. Matings involving blues are not included in this Table, and blue offspring are included as blacks. Blues breed as blacks except that blue × blue should never throw blacks. Aberrant litters are not included here (see Table I), and depleted litters are only included if one parent was known to be homozygous black EE.

Parents.	No. of Litters.	Offspring.		Remarks.
1. BLACK × BLACK.		EXPECT BLACKS and EITHER BRINDLES OR FAWNS, not both.		
Genotype.		Offspring expected.	Offspring obtained.	
(a) EE × E -	6	All black	31 Bk + 3d	See p. 465.
(b) EE' × EE' or Ee	15	3 Bk : 1 Bd	80 Bk : 28 Bd	No litters of brindles only.
(c) Ee × Ee	7	3 Bk : 1 F	33 Bk : 12 F	No litters of fawns only.
2. BLACK × BRINDLE.		EXPECT BLACKS alone or with BRINDLES AND FAWNS.		
(a) EE × E' -	9	All black	56 Bk + 7d	See p. 465.
(b) EE' × E' -	77	1 Bk : 1 Bd	226 Bk : 227 Bd : 2 W	7 litters contain Bd only (26 pups).
(c) Ee × E'e	65	2 Bk : 1 Bd : 1 F	180 Bk : 123 Bd : 140 F	6 litters contain Bd and F only (42 pups), and 6 litters contain Bk and F only (28 pups).
3. BLACK × FAWN.		EXPECT BLACKS and EITHER BRINDLES OR FAWNS, not both.		
(a) EE × ee	8	All black.	31 Bk + 8d	See p. 465.
(b) EE' × ee	31	1 Bk : 1 Bd	96 Bk : 121 Bd	2 litters contain Bd only (8 pups).
(c) Ee × ee	56	1 Bk : 1 F	185 Bk : 187 F	2 litters contain F only (8 pups).

Parents.	No. of Litters.	Offspring.	Remarks.
4. BRINDLE × BRINDLE. EXPECT BRINDLES alone or with FAWNS.			
(a) E'E' × E' -	78	All brindle. 481Bd	
(b) E'e × E'e	116	3Bd : 1F 513Bd : 235F	1 litter contains F only (1 pup).
5. BRINDLE × FAWN. EXPECT BRINDLES alone or with FAWNS.			
(a) E'E' × ee	41	All brindles. 249Bd	
(b) E'e × ee	223	1Bd : 1F 757Bd : 711F	15 litters contain F only (62 pups).
6. FAWN × FAWN. EXPECT ONLY FAWNS.			
	82	.. 537F	Note 31 aberrant litters.

TABLE III.—INHERITANCE OF FAWN, RED-FAWN, AND RED.

Cross.	Litters Total.	Number of Litters containing			
		F only.	RF only.	R only.	F plus RF and/or R.
F × F	26	17	2	..	7 (24F : 11RF : 3R pups).
F × RF	27	14	6	1	6 (21F : 23RF : 2R pups).
F × R	2	2 (4F : 4RF : 3R pups).
RF × RF or R	26	4	14	2	6 (16F : 11RF : 9R pups).
R × R	1	1 (3F : 1RF pups).

TABLE IV.—RELATIONS OF FAWN AND RED: ANALYSIS OF FAWN OFFSPRING FROM MATINGS WITH BLACK AND BRINDLE.

Cross.	Litters Total.	F only.	Litters containing	
			RF and R only.	Both F and RF and/or R.
(a) <i>Fawn × Black and Brindle.</i>				
Bk × F	8	4	3	1
F × Bk	4	2	1	1
Bd × F	40	29	6	5
F × Bd	17	12	1	4
Total	69	47	11	11
(b) <i>Red and Red-Fawn × Black and Brindle.</i>				
Bk × RF	13	3	6	4
RF × Bk	15	6	8	1
Bd × RF	40	19	16	5
RF × Bd	35	9	19	7
Total	103	37	49	17

TABLE V.—INHERITANCE OF WHITE SPOTTING.

(Including data from matings of black with black, blue, and brindle, and the reciprocal matings only.)

Cross.	Offspring (F indicates red or fawn pups; main figure includes only black, blue, and Hd pups).						
	Self.		Predom. Col.		Predom. White.		White.
Self × Self	331	61F	157	13F	15	1F	..
Self × Predom. Col.	161	32F	192	16F	23	5F	..
Self × Predom. White	24		36	4F	8	2F	1
Self × White	2		1		4		1
Predom. Col. × Predom. Col.	21	1F	94	3F	36	2F	..
Predom. Col. × Predom. White	6		13		10		..
Predom. Col. × White	..		4		3		..
Predom. White × Predom. White	..		1		18		..
Predom. White × White	4		1		2		..
White × White	No mating						

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DESCRIPTION OF PLATE.

The photomicrographs are all from whole hairs cleared and mounted as described in text, and are all on the same scale as indicated. They were taken with one-sixth objective and green screen.

Fig. 1. Black overhair, near tip. Heavily pigmented, medulla tapering.

Fig. 2. Red overhair, near tip. Many small pigment granules in cortex and between air-spaces in medulla.

Fig. 3. Blue overhair, about middle. Shows scattered granules and aggregations of pigment in cortex, and deformed medulla.

Fig. 4. Black overhair, near tip. After bleaching in hydrogen peroxide solution (see text).

Fig. 5. Red overhair, near tip. After bleaching in hydrogen peroxide solution.

Fig. 6. Blue overhair, about middle. After bleaching in hydrogen peroxide solution.

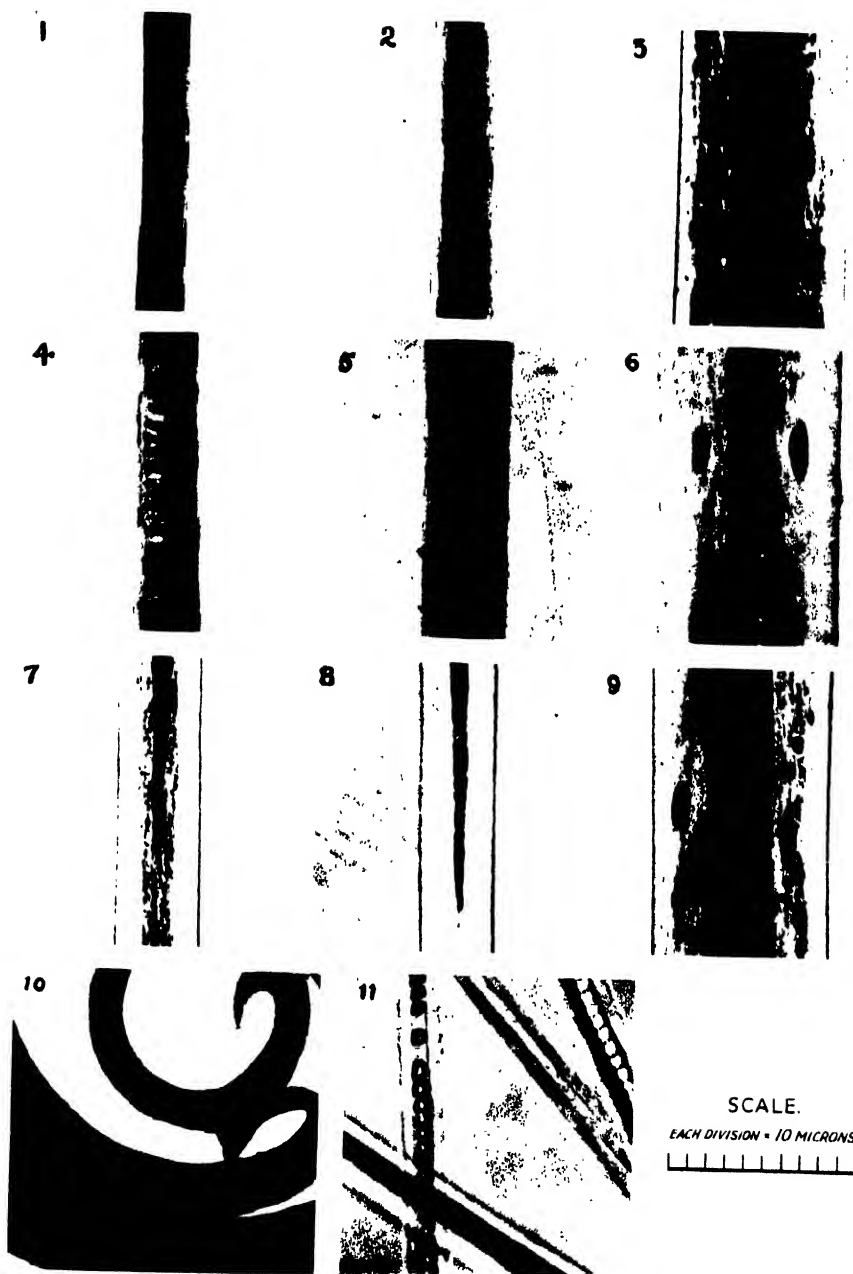
Fig. 7. Black overhair, near base. Shows basal end of medulla and scattered pigment granules.

Fig. 8. Red overhair, near base. Shows basal end of medulla (not cleared); no pigment granules in this region.

Fig. 9. Fawn hair from blue-fawn-brindle hound. Middle region. Shows pigment granules and aggregations of two shades (red and sepia).

Fig. 10. Black hair after immersion in strong potassium hydroxide solution. Hair curled, but no reduction of pigment.

Fig. 11. White hairs: an overhair and three wool hairs. To show general structure, and optical effect of partial clearance of medulla. Cuticular scales partly visible. No pigment granules.



INDEX

- Acrosome Formation in Mouse, by R. A. R. Gresson, 197-210.
- Ahmed (I. A.). Cytological Analysis of Chromosome Behaviour in Three Breeds of Dogs, 107-118.
- Ammonite, Growth Stages in, by E. D. Currie, 344-367.
- Amylase Activity, Time and pH Relations in, by J. Small and J. Crosbie-Baird, 254-266.
- Antennal Gland, *Marinogammarus marinus*, Sphincter Valve of, by M. V. Schorstein, 130-137.
- Apparatus for the Direct Determination of the Volume of Small Irregular Objects, by B. N. Smallman, 186-187.
- Bacsich (P.) and Wyburn (G. M.). Observations on Artificially Induced Heat in Immature Guinea-Pigs, 188-196.
- Burns (Marca). Hair Pigmentation and the Genetics of Colour in Greyhounds, 462-490.
- Calanus finmarchicus*, Feeding and Breeding of, under Laboratory Conditions, by J. E. G. Raymont and F. Gross, 267-287.
- Specific Gravity of, by F. Gross and J. E. G. Raymont, 288-296.
- Cardiopteridium*, a Genus of Fossil Plants of Lower Carboniferous Age, by J. Walton, 59-66.
- Cat, Sex Chromosomes in the, by P. C. Koller, 78-94.
- Chromosomes in the Cat, by P. C. Koller, 78-94.
- in Three Breeds of Dogs, by I. A. Ahmed, 107-118.
- Types and Development of Polytene, by A. M. Melland, 316-327.
- Clarke (M. I.). See Small (J.), Crosbie-Baird (J.), and Clarke (M. I.).
- Colour in Greyhounds, Hair Pigmentation and Genetics of, by Marca Burns, 462-490.
- Continents, Evolution of, by Sir T. H. Holland, 149-166.
- Cosmetira pilosella*, Hydroid of, by W. J. Rees, 55-58.
- newly liberated from Hydroid, by W. J. Rees, 55-58.
- Crosbie-Baird (J.). See Small (J.), Crosbie-Baird (J.), and Clarke (M. I.).
- Currie (E. D.). Growth Stages in the Ammonite *Promicroceras marstonense* Spath., 344-367.
- Deficiency Effects of Ultra-Violet Light in *Drosophila melanogaster*, by B. M. Slizynski, 297-315.
- Diptera, Salivary Glands and Chromosomes in, by A. M. Melland, 316-327.
- Dixippus (Carausius) morosus*, Br. et Redt., Quantitative Characters of the Growth and Development. I. Loss of Water in relation to Ecdysis, by B. N. Smallman, 167-185.
- Dog, Chromosomes in the, by I. A. Ahmed, 107-118.
- Drosophila*, Mutation and Lethal Effects of Ultra-Violet Irradiation on, by K. MacKenzie, 67-77.
- Structural Differentiation of Chromosome IV in, by B. M. Slizynski, 95-106.
- *melanogaster*, Deficiency Effects of Ultra-Violet Light in, by B. M. Slizynski, 297-315.
- *melanogaster* and *simulans*, Hybrid Sterility in, by G. Pontecorvo, 385-397.
- Duct, Intermediate Character of, by M. V. Schorstein, 375-384.
- Ecdysis, Loss of Water in relation to, in *Dixippus (Carausius) morosus*, Br. et Redt., by B. N. Smallman, 167-185.
- Edington (G. H.) and Miller (Agnes E.). The Avian Ulna: Its Quill-Knobs, 138-148.
- Endodermis, The Occurrence and Effect of, in Leguminous Root Nodules, by H. L. Frazer, 328-343.
- Etherington (I. M. H.). Non-Associative Algebra and the Symbolism of Genetics, 24-42.
- Euphausiid, Larval Segmental Excretory Organs in, by M. V. Schorstein, 375-384.
- Evolution of Continents, by Sir T. H. Holland, 149-166.
- Feeding and Breeding of *Calanus finmarchicus* under Laboratory Conditions, by J. E. G. Raymont and F. Gross, 267-287.
- Frazer (H. L.). The Occurrence of Endodermis in Leguminous Root Nodules and its Effect upon Nodule Function, 328-343.
- Genetics, Symbolism of, by I. M. H. Etherington, 24-42.
- Golden Hamster, Breeding outside the Season after Injection of Gonad-stimulating Hormone, by O. Peczenik, 368-374.
- Golgi Material in Middle-piece of Sperm of Mouse, by R. A. R. Gresson, 197-210.

- Gonad-stimulating Hormone, Influence on Breeding Pause of the Golden Hamster, by O. Peczenik, 368-374.
- Graham (A.). On the Œsophagus of the Stenoglossan Prosobranchs, 1-23.
- Gresson (R. A. R.). A Study of the Cytoplasmic Inclusions during the Spermatogenesis of the Mouse, 197-210.
- Greyhounds, Hair Pigmentation and the Genetics of Colour in, by Marca Burns, 462-490.
- Gross (F.) and Raymont (J. E. G.). The Specific Gravity of *Calanus finmarchicus*, 288-296.
- Gross (F.). See Raymont (J. E. G.) and Gross (F.).
- Growth and Development of a Paurometabolous Insect, *Dixippus (Carausius) morosus*, Br. et Redt. I: by B. N. Smallman, 167-185.
- Growth Stages in *Promicroceras marstonense*, by E. D. Currie, 344-367.
- Gryphaea incurva* (Sow.), Variation in, by Rhona M. MacLennan and A. E. Trueman, 211-232.
- Guinea-Pigs, Immature, Artificially Induced Heat in, by P. Bacsich and G. M. Wyburn, 188-196.
- Harland (W. B.). Geological Notes on the Stubendorff Mountains, West Spitsbergen, 119-129.
- Heat, Artificially Induced, in Immature Guinea-Pigs, by P. Bacsich and G. M. Wyburn, 188-196.
- Holland (Sir T. H.). The Evolution of Continents: a Possible Reconciliation of Conflicting Evidence, 149-166.
- Hybrids, Salivary Gland Studies of Chromosome IV in Partial, by B. M. Slizynski, 95-106.
- Irregular Objects, Small, A Simple Apparatus for the Direct Determination of the Volume of, by B. N. Smallman, 186-187.
- Kevan (D. Keith McE.). Study of an Introduced North American Freshwater Mollusc, *Stagnicola catascopium* (Say), 430-461.
- Koller (P. C.). The Genetical and Mechanical Properties of the Sex Chromosomes. VIII. The Cat (*Felis domestica*), 78-94.
- The Effects of Radiation on Pollen Grain Development, Differentiation and Germination, 398-429.
- Lethal Effects of Ultra-Violet Irradiation on *Drosophila*, by K. Mackenzie, 67-77.
- McCallien (W. J.). The Structure of Tulach Hill, Blair Atholl, Perthshire, 43-54.
- Mackenzie (K.). Mutation and Lethal Effects of Ultra-Violet Irradiation on *Drosophila*, 67-77.
- MacLennan (Rhona M.) and Trueman (A. E.). Variation in *Gryphaea incurva* (Sow.) from the Lower Lias of Loch Aline, Argyll, 211-232.
- Marinogammarus marinus*, Sphincter Valve of the Antennal Gland of, by M. V. Schorstein, 130-137.
- Melland (A. M.). Types and Development of Polytene Chromosomes, 316-327.
- Miller (Agnes E.). See Edington (G. H.) and Miller (Agnes E.).
- Mutation in *Drosophila*, by Ultra-Violet Irradiation, by K. Mackenzie, 67-77.
- Non-Associative Algebra and the Symbolism of Genetics, by I. M. H. Etherington, 24-42.
- Œsophagus of the Stenoglossan Prosobranchs, by A. Graham, 1-23.
- Peczenik (O.). Induction of Pregnancy in the Golden Hamster during the Breeding Pause, 368-374.
- pH Phenomena in relation to Stomatal Opening. II-V: by J. Small, M. I. Clarke, and J. Crosbie-Baird, 233-266.
- Pigmentation, Hair, and the Genetics of Colour in Greyhounds, by Marca Burns, 462-490.
- Pollen Grain, Effects of Radiation on, by P. C. Koller, 398-429.
- Pontecorvo (G.). Hybrid Sterility in Artificially Produced Recombinants between *Drosophila melanogaster* and *D. simulans*, 385-397.
- Promicroceras marstonense*, Growth Stages in, by E. D. Currie, 344-367.
- Quill-Follicle Ligaments, by G. H. Edington and Agnes E. Miller, 138-148.
- Raymont (J. E. G.) and Gross (F.). On the Feeding and Breeding of *Calanus finmarchicus* under Laboratory Conditions, 267-287.
- Raymont (J. E. G.). See Gross (F.) and Raymont (J. E. G.).
- Rees (W. J.). The Hydroid of the Medusa *Cosmetira pilosella* Forbes, 55-58.
- Root Nodules, Leguminous, The Occurrence and Effect of Endodermis in, by H. L. Frazer, 328-343.
- Schorstein (M. V.). On the Sphincter Valve of the Antennal Gland of *Marinogammarus marinus*, 130-137.
- The Larval Maxillary Glands of *Euphausia superba* Dana, 375-384.
- Shell-form, Variation in, of Introduced North American Mollusc, *Stagnicola catascopium* (Say) under abnormal (thermal) conditions, by D. Keith McE. Kevan, 430-461.
- Slizynski (B. M.). The Structural Differentiation of Chromosome IV of *Drosophila simulans* and its Behaviour in *melanogaster* Genotype, 95-106.

- Slizynski (B. M.). Deficiency Effects of Ultra-Violet Light in *Drosophila melanogaster*, 297-315.
- Small (J.), Clarke (M. I.), and Crosbie-Baird (J.). pH Phenomena in relation to Stomatal Opening. II-V, 233-266.
- Smallman (B. N.). Quantitative Characters of the Growth and Development of a Paurometabolous Insect, *Dixippus (Curausius) morosus*, Br. et Redt. I. The Loss of Water in relation to Ecdysis, 167-185.
- A Simple Apparatus for the Direct Determination of the Volume of Small Irregular Objects, 186-187.
- Specific Gravity of *Calanus finmarchicus*, by F. Gross and J. E. G. Raymont, 288-296.
- Spermatogenesis, Cytoplasmic Inclusions in Mousc, by R. A. R. Gresson, 197-210.
- Sphincter Valve, Ectodermal Origin of, by M. V. Schorstein, 375-384.
- Striated Myofibrils within Cells of, by M. V. Schorstein, 130-137.
- Spitsbergen, Part Results of Cambridge S. Expedition (1938), by W. B. Harland, 119-129.
- Stagnicola catascopium* (Say). Study of Introduced North American Mollusc, by D. Keith McE. Kevan, 430-461.
- Stenoglossan Prosobranchs, (Esophagus of, by A. Graham, 1-23.
- Sterility, Hybrid, in *Drosophila melanogaster* and *simulans*, by G. Pontecorvo, 385-397.
- Stomatal Movements, Time Relations of, by J. Small and M. I. Clarke, 247-254.
- Stomatal Opening, pH Phenomena in relation to. II-V, by J. Small, M. I. Clarke, and J. Crosbie-Baird, 233-266.
- Stubendorff Mountains, Geological Structure of, by W. B. Harland, 119-129.
- Trueman (A. E.). See MacLennan (Rhona M.) and Trueman (A. E.).
- Tulach Hill, Blair Atholl, Perthshire, Structure of, by W. J. McCallien, 43-54.
- Ulna, Avian, Additional Markings in, by G. H. Edington and Agnes E. Miller, 138-148.
- Ultra-Violet Light, Deficiency Effects of, in *Drosophila melanogaster*, by B. M. Slizynski, 297-315.
- Ultra-Violet, Mutation and Lethal Effects on *Drosophila*, by K. Mackenzie, 67-77.
- Variation in *Gryphaea incurva* (Sow.) from the Lower Lias of Loch Aline, Argyll, by Rhona M. MacLennan and A. E. Trueman, 211-232.
- Variation in Shell-form under Abnormal (Thermal) Conditions of Introduced North American Mollusc, *Stagnicola catascopium* (Say), by D. Keith McE. Kevan, 430-461.
- Walton (J.). On *Cardiopteridium*, a Genus of Fossil Plants of Lower Carboniferous Age, with Special Reference to Scottish Specimens, 59-66.
- Wyburn (G. M.). See Bacsich (P.) and Wyburn (G. M.).
- X-ray—Effects on Pollen Grain Development, Differentiation and Germination, by P. C. Koller, 398-429.

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